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Fisheries Research Board of Canada
Office of the Editor
116 Lisgar Street
Ottawa 4, Ont., Canada

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Description of Laboratory-Reared Larvae of *Paralithodes platypus* (Decapoda, Anomura, Lithodidae)[1]

By Ethelwyn G. Hoffman[2]

U.S. Bureau of Commercial Fisheries
Biological Laboratory, Auke Bay, Alaska

ABSTRACT

Larvae of the blue king crab, *Paralithodes platypus* (Brandt), were hatched and reared in the laboratory. All larval stages obtained developed in a manner similar to the development reported for other lithodid anomurans. In culture, *P. platypus* had four zoeal stages and a single glaucothoeal stage. The feature which distinguishes all zoeal stages of this species from zoeae of the other two North Pacific species of *Paralithodes* — *P. camtschatica* and *P. brevipes* — is the presence of 9 + 9 telson processes (including a hair-like second process) rather than 8 + 8 telson processes. Glaucothoes of *P. platypus* have one more pair of spines in the branchial region of the carapace than do those of *P. camtschatica*. Glaucothoes of *P. platypus* have 15 pairs of spines on the dorsal surface of the carapace, not including the spines of the frontal area (rostral complex) or the suborbital spines, whereas glaucothoes of *P. camtschatica* have 14 pairs of spines, and those of *P. brevipes* have 13 pairs.

INTRODUCTION

Paralithodes platypus (Brandt), called the blue king crab in Alaska, is an anomuran crustacean of the family Lithodidae. The genus *Paralithodes* has five species, three of which — *P. camtschatica*, *P. brevipes*, and *P. platypus* — have broadly overlapping distributions in the northern half of the North Pacific Ocean. In the west, the range of *P. platypus* extends from the coast of Hokkaido, Japan, northward along the eastern shores of the Kamchatka Peninsula and into the southern Bering Sea (Makarov, 1938). Farther east this species is found south of the Aleutian Islands, around Kodiak Island, and along the coast of the Gulf of Alaska. The most southerly point of its distribution on the eastern Pacific coast appears to be Gambier Bay on Admiralty Island in southeastern Alaska (57°27′N lat, 133°53′W long) (Carl Lehman, Alaska Department of Fish and Game, personal communication).

The most comprehensive review of the literature on the larval development of lithodid crabs appears in Hart's (1965) paper describing the life history and larval development of *Cryptolithodes typicus*. Hart's review does not include the nearly concurrent publication by Kurata (1964) of a comprehensive description of the developmental stages of lithodid larvae of Hokkaido, Japan, including a key for their identification. Kurata described the first zoeal stage of *P. platypus* and all the larval stages of *P. camtschatica* and *P. brevipes*.

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[1]Received for publication May 24, 1967.
[2]Present address: Box 615, Shelton, Wash. 98584.

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Of the three species of *Paralithodes* found in the northern part of the North Pacific, two — *P. camtschatica* and *P. brevipes* — have been reared in the laboratory through all of their larval stages. The first complete description the larval stages of *P. camtschatica* was by Marukawa (1933). The source of zoeal stages I and II was eggs hatched in the laboratory, and that of later stages was the plankton. Sato (1958) also discussed the development of larvae of *P. camtschatica*. Experimental work on the larvae of this species was carried out by Kurata. He studied rearing techniques, kinds of organisms eaten and the rate of their consumption by king crab larvae (Kurata, 1959, 1960a), and the effect of temperature and salinity on growth and survival of larvae (Kurata, 1960b). A larva intermediate between the normal last zoea and the glaucothoe was described for *P. camtschatica* and *P. brevipes* from specimens reared in the laboratory (Kurata, 1960c). Kurata (1956) reared *P. brevipes* and found that this species has only three zoeal stages instead of the four formerly assumed to characterize the genus. Both *P. camtschatica* and *P. brevipes* have a single glaucothoeal stage, as do all lithodids described to date (Sato, 1958; Kurata, 1964; Hart, 1965).

Larvae of *P. platypus* have been the least thoroughly described. Marukawa (1933) illustrated the first zoeal stage but because he made no mention of rearing these larvae, it is assumed that they were taken from the plankton. Sato (1958) illustrated zoeal stages I–IV but gave no detailed descriptions. The illustration of the first zoeal stage was based on Marukawa's work, and those of stages II–IV were made from individuals collected from the intestinal tract of juvenile pink salmon. Thus, the source of Sato's material was not larvae reared in culture as Hart (1965) had assumed. Kurata (1964) described stage I zoeae taken from the plankton. This larval stage was the only one that he obtained. Kurata briefly discussed stages II–IV, basing his comments on Sato's (1958) work.

Because of their importance, I obtained English translations of the relevant parts of the papers by Sato (1958) and Kurata (1964) and for all of Marukawa (1933). I used only the English summaries and the illustrations in the other Japanese papers cited.

The previous descriptions of morphology of the larval stages of *P. platypus* were based on specimens from the plankton and the contents of pink salmon stomachs. Species identity could not be known positively. In the present study a gravid female *P. platypus* with hatching eggs was brought into the laboratory where the larvae were collected and reared. The resulting four zoeal and single glaucothoeal stages are described.

The larvae reared during this study were sent to Dr J. F. L. Hart of Victoria, B.C., for final disposition in the British Columbia Provincial Museum.

METHODS AND MATERIALS

On April 7, 1965, I captured a clasping pair of *P. platypus* while I was skin diving in Auke Bay, Alaska (58°21′N lat, 134°14′W long). The female was ovigerous, and the eggs were hatching. The length of her carapace was

149.5 mm from the posterior margin of the right orbit to the posterior medial margin of the carapace, and the width was 161.5 mm across the widest part of the carapace, not including the spines.

Zoeae were obtained and cultured in the following manner. The gravid female was held in a large aquarium with continuous running sea water. The eggs were allowed to hatch naturally, which precluded observing prezoeae. The first zoeae were removed from the aquarium with a large-bore pipette and placed in glass finger bowls (5 mm deep × 8 mm in diam). Initially six zoeae were placed in each bowl. Additional larvae for preservation at various stages were kept in large glass beakers. The larvae in the finger bowls were examined daily, and signs of molting, disease, or death were recorded. The duration of the larval stages was determined from these observations.

Nauplii of brine shrimp (*Artemia* sp.) were added to the bowls daily as food for the larvae. In addition, the glaucothoes also received small amounts of minced clams, *Protothaca* sp., on which they appeared to feed.

The water used in this study was taken from the natural environment. The static water in the culture bowls was changed every second day. The culture dishes were kept in a circulating seawater bath whose temperature increased gradually from 4.5 to 7.2 C (40.1–45.0 F) during the 2½-month study. The increase in temperature of the bath water corresponded to the seasonal increase in temperature of the bay from which the sea water was taken. The salinity of the cultures varied between 30.0 and 32.0‰.

Drawings of appendages were made from exuviae from the finger bowls, and drawings of whole animals were made from preserved specimens from the mass cultures in the beakers. All illustrations were prepared with the aid of a camera lucida. The left appendage was drawn unless stated otherwise. When setae were plumose, the fact is noted in the text but usually is not shown in the drawings. This omission made it possible to show other details. Exuviae and preserved animals were cleared in lactic acid and were drawn while immersed in this medium (Humes and Gooding, 1964).

An ocular micrometer was used to measure body dimensions of the larvae — carapace length, total body length, and total carapace length. *Carapace length* is the straight-line distance from the posterior margin of the orbit to the mid-dorsal posterior margin of the carapace. Carapace length is the standard measurement in this study because it is the least subject to variations caused by preservation and handling and also because it is generally used in studies of juvenile and adult crabs. *Total body length* is the distance from the tip of the rostrum to the posterior margin of the telson, not including the telson processes. An accurate measurement of total body length is difficult to obtain in preserved larvae because the abdomen is generally flexed tightly beneath the carapace. This measurement requires the summation of several straight-line measurements or chords of the arch formed by the dorsal margin of the body. *Total carapace length* of the zoea is the straight-line distance from the tip of the rostrum to the tip of the lateral spine. In the glaucothoe, total carapace length is measured from the tip of the rostrum to the posterior mid-

dorsal margin because of a change in the form of the carapace. Data on average total body length and total carapace length are presented to facilitate comparison with similar measurements given in the literature. The *width of the carapace* of the glaucothoe is the distance across the widest part of the branchial region between spines.

Nomenclature of the larval appendages follows that of Gurney (1942), except when noted. The term "telson process," proposed by Pike and Williamson (1960), includes spines, setae, or hairs on the telson. The setation formula of the endopodite is the numbers of setae per segment from the distal to the proximal segment.

GENERAL REARING DATA

Forty-eight stage I zoeae were placed in glass bowls (six per bowl). Table I shows the number of individuals surviving to the beginning of each larval stage, the duration of each stage, and the accumulated mortality (expressed

TABLE I. Duration of the larval stages (days) and cumulative mortality (per cent) through each stage of *Paralithodes platypus* reared in the laboratory.

Larval stage	Larvae entering each stage	Duration		Cumulative mortality through each stage
		Avg	Range	
Zoea I	48	12.4	10–15	75
Zoea II	12	12.3	11–14	77.1
Zoea III	11	12.5	11–15	83.3
Zoea IV	8	14.3	13–17	87.5
Glaucothoe	6	12.8[a]	9–18[b]	100
Post-larvae	0	–	–	–

[a]No glaucothoes molted to the first post-larval stage. Since most of the deaths of the larvae occurred just before ecdysis, the date of death was assumed to be the date of ecdysis.
[b]The range is the number of days the glaucothoe lived.

as the percentage of the original larvae that had died by the end of each larval stage). Mortality was greatest late in zoeal stage I, when some individuals died apparently without starting ecdysis and others without completing ecdysis or exuviation.

Under the conditions of this study, *P. platypus* went through four zoeal stages and molted into the glaucothoeal stage. None of the glaucothoes molted to the post-larval stage. It is assumed that *P. platypus* has a single glaucothoeal stage, as in all other species of Lithodidae described to date.

DESCRIPTIONS OF LARVAL STAGES

STAGE I ZOEA

The average carapace length (from eye notch to mid-dorsal margin of carapace) of stage I zoeae (Fig. 1*a*) is 1.2 mm (42 specimens); the average total carapace length (from tip of rostrum to tip of lateral spine) is 3.2 mm (10 specimens); and the average total body length (from tip of

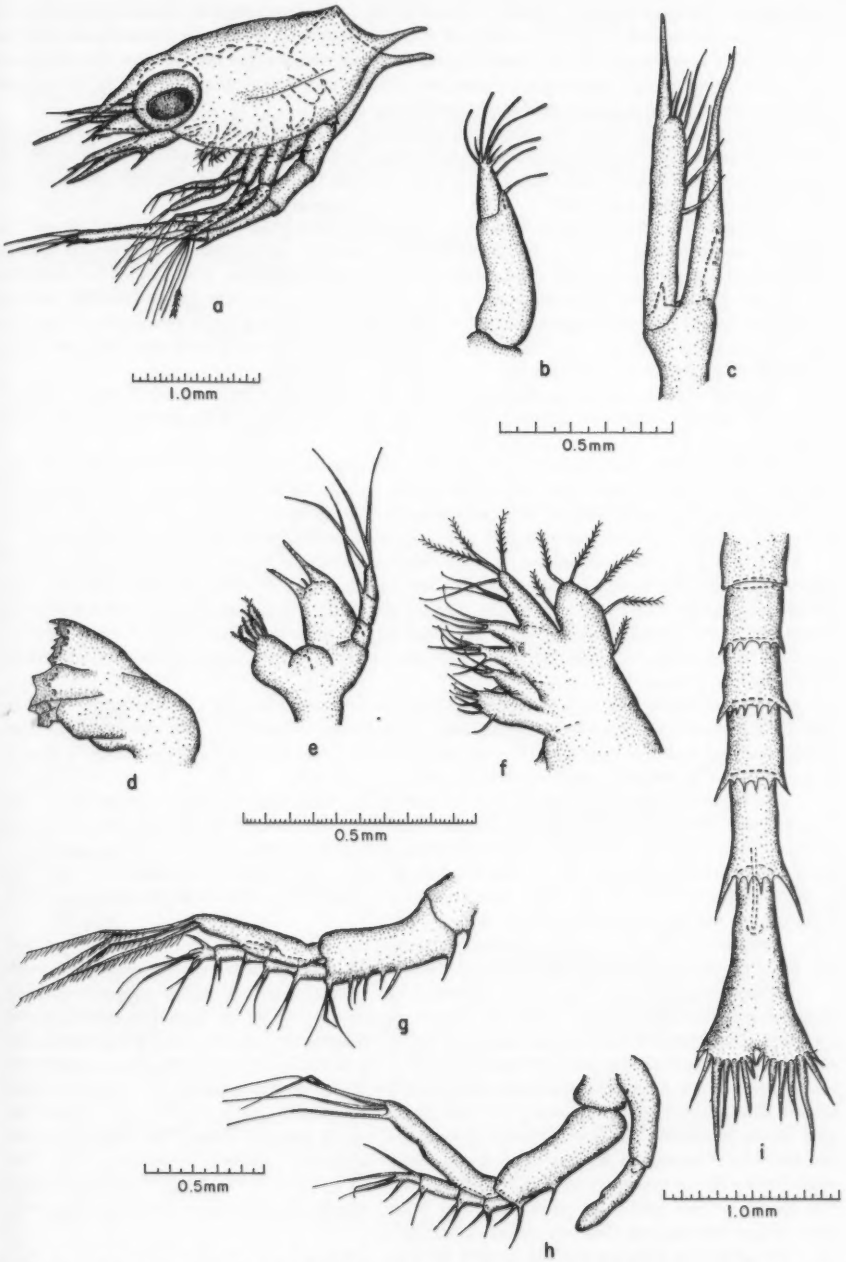

FIG. 1. (*a*) Stage I zoea of *Paralithodes platypus*; (*b*) antennule; (*c*) antenna; (*d*) mandible, right; (*e*) maxillule, right; (*f*) maxilla, right; (*g*) first maxilliped; (*h*) second and third maxillipeds; (*i*) abdomen and telson.

rostrum to posterior margin of telson, not including telson processes) is 4.9 mm (7 specimens). Lateral carinae extend toward the spines on the postero-lateral margin of the carapace and are present in all zoeal stages. In all zoeal stages the carapace has a slight medial crest, which bears a noticeable hump at the base of the rostrum, and the rostrum is approximately one-third the total carapace length. The eyes are sessile in stage I only.

Antennule (*Fig. 1b*). — The outer distal segment of the antennule has seven aesthetes and two small setae. The proximal segment has no setae, although this segment has an extension or distal lobe on the medial side. This lobe is slightly more than one-third and less than one-half the length of the distal segment.

Antenna (*Fig. 1c*). — The antenna has a basal segment which bears two stout spines. The medial spine is segmented from the basis, and the distal half is denticulate. The lateral spine is smaller and smooth and is not segmented from the basal segment. The exopodite (antennal scale) has eight sparsely plumose setae on the medial margin and terminates in a long slender projection which extends beyond the end of the endopodite. The narrow endopodite is not segmented from the basis and terminates in a finely bifid tip. The exopodite and endopodite are subequal in length in all zoeal stages.

Mandible (*Fig. 1d, right mandible*). — The mandible has a series of small teeth dorsally and larger teeth on the molar process. There is no indication of a mandibular palp in any zoeal stage.

Maxillule (Fig. *1e, right maxillule*). — The endopodite (palp of Hart, 1965) is three-segmented; it has three setae on the terminal segment, one on the middle segment, and none on the basal segment. The distal end of the basal endite has two short spines and two large spines; each of the latter bears a row of spinules. The proximal coxal endite has six sparsely plumose setae.

Maxilla (*Fig. 1f, right maxilla*). — The simple and lobular exopodite has six widely spaced plumose setae. The unsegmented endopodite has four sparsely plumose terminal setae and two subterminal setae. There are two endites. The basal endite (distal) has four and five setae on its distal and proximal lobes respectively. The coxal endite (proximal) has four and eight setae on its distal and proximal lobes respectively. The maxillule and maxilla are described and illustrated only for stage I zoeae.

First maxilliped (*Fig. 1g*). — The exopodite has four long, heavily plumose setae. Setae on the exopodites of the maxillipeds, when present, are always terminal. The endopodite is five-segmented and equal in length to the exopodite. The setation formula of the endopodite is 6, 2, 2, 5, 2. The basis has nine small setae on its ventral surface.

Second maxilliped (*Fig. 1h*). — The exopodite has four long, heavily plumose setae. The four-segmented endopodite is slightly shorter than the exopodite. The setation formula of the endopodite is 5, 2, 2, 2. The basis has three setae ventrally on the distal half of the segment.

Third maxilliped (*Fig. 1h*). — The third maxilliped is composed of two segments, the basis and the exopodite, and has no setae. The endopodite is evident only as a slight extension of the basis and is not segmented from the basis.

Pereiopods (*Fig. 1a*). — The developing pereiopods are present beneath the carapace, and the tip of the first pereiopod is already bifid. None of the pereiopods are segmented at this stage.

Abdomen and telson (*Fig. 1i*). — Segments 2–5 have one pair of lateral and two pairs of dorsal spines on their posterior margins. Some variation exists in the dorsal spination of the abdomen. For example, an "extra," unpaired spine is sometimes present. The lateral spines are progressively longer on the more posterior segments. Those of the fifth segment extend posteriorly to the level of the anus. The posterior margin of the telson is cleft medially to form the slight lateral lobes characteristic of many lithodid larvae. Terminally, each of the paired lobes has nine telson processes. The first (lateral) process, a spine, is not articulated; the second is small and hair-like. This second process was first recorded in zoeae of *P. platypus* by Kurata (1964). The medial seven telson processes on each lobe are articulated spines with small denticulations basally. The fourth process (the third spine) from the lateral margin of each telson lobe is at least one-third longer than any of the other telson processes.

Pleopods and uropods are not evident on stage I zoeae.

The number of segments and their spination, as well as the number and distribution of the telson processes are the same in all zoeal stages. Because the changes are few and the descriptions long, the telson processes are not described for each stage.

STAGE II ZOEA

At this stage (Fig. 2*a*) the average carapace length is 1.3 mm (33 specimens); the average total carapace length is 3.4 mm (9 specimens); and the average total body length is 5.2 mm (10 specimens). The eyes are stalked and movable in this and all subsequent stages.

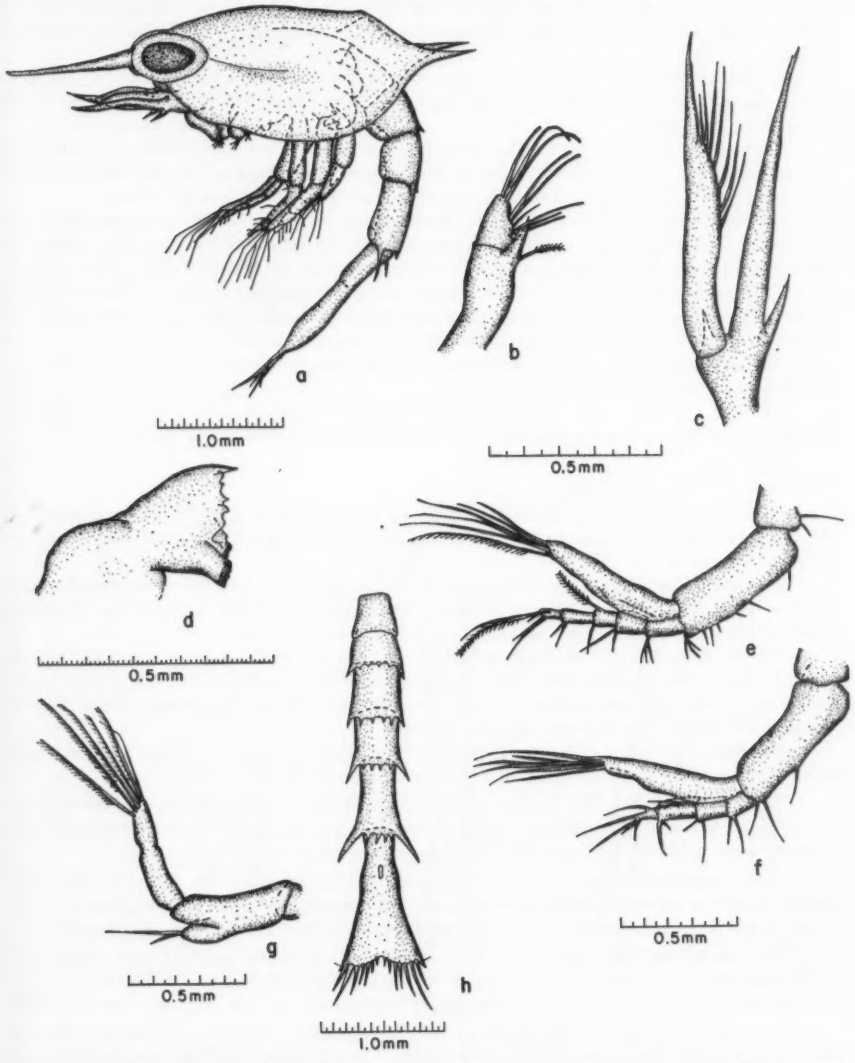

FIG. 2. (*a*) Stage II zoea of *P. platypus*; (*b*) antennule; (*c*) antenna; (*d*) mandible; (*e*) first maxilliped; (*f*) second maxilliped; (*g*) third maxilliped; (*h*) abdomen and telson.

Antennule (*Fig. 2b*). — The outer distal segment of the antennule has eight aesthetes and at least two setae. Terminal setae are lacking on the lobe of the proximal segment, although in some individuals a single sparsely seta lies at the base of this lobe. The lobe is slightly longer than one-half the length of the distal segments.

Antenna (*Fig. 2c*). — The antenna is similar to that of stage I zoeae. The basal segment has two stout spines. Only the medial one is segmented from the basis, and this spine has small spinules on its distal half. The lateral spine is the smaller and is smooth. The exopodite has eight sparsely plumose setae on the medial margin and terminates in a long, slender projection which extends beyond the end of the endopodite. The narrow endopodite is not segmented from the basis, and the tip of the endopodite is slightly bifid. The exopodite and endopodite are of subequal length.

Mandible (*Fig. 2d*). — The antero-dorsal portion of the mandible has several teeth dorsally. The molar process, which had large teeth in stage I, now consists of three sections, each with numerous tubercles.

First maxilliped (*Fig. 2e*). — The exopodite has six long, heavily plumose terminal setae. The endopodite is five-segmented and equal in length to the exopodite. The setation formula of the endopodite is 5, 2, 2, 3, 4. The basis has nine small setae on its ventral surface.

Second maxilliped (*Fig. 2f*). — The exopodite has six long, heavily plumose setae. The endopodite is four-segmented and slightly shorter than the exopodite. The setation formula of the endopodite is 5, 3, 3, 2. The basis has three setae ventrally on the distal half of the segment.

Third maxilliped (*Fig. 2g*). — The exopodite has six plumose terminal setae. The endopodite arises from the side of the basis and does not extend beyond the basis. Terminally, the endopodite has one long and one short seta. The endopodite is not segmented from the basis.

Pereiopods (*Fig. 2a*). — The pereiopods are larger than in stage I but have the same form. The tip of the first pereiopod is bifid.

Abdomen and telson (*Fig. 2h*). — The abdomen and telson are similar to those of stage I. Tissue buds of the developing pleopods and uropods can be seen within the exoskeleton.

Stage III Zoea

Stage III zoeae (Fig. 3*a*) have an average carapace length of 1.6 mm (10 specimens); an average total carapace length of 3.5 mm (5 specimens); and an average total body length of 5.5 mm (5 specimens).

Antennule (*Fig. 3b*). — The distal segment of the antennule has eight aesthetes and two or three setae. The medial extension or lobe of the proximal segment is about two-thirds the length of the distal segment and has a single plumose seta at its base.

Antenna (*Fig. 3c*). — The shape, spination, and setation of the antenna resemble those of stages I and II. The basal segment has two stout spines. Only the medial one is segmented from the basis, and this spine has small spinules on its distal half. The smaller lateral spine is smooth. The exopodite and endopodite are subequal in length.

Mandible (*Fig. 3d*). — The mandible is similar to that of stage II. It has a well-developed molar process. There is no indication of a developing mandibular palp.

First maxilliped (*Fig. 3e*). — The exopodite has six long, heavily plumose terminal setae. The endopodite is five-segmented and equal in length to the exopodite. The setation formula of the endopodite is 5, 2, 2, 3, 4. The basis has nine small setae on its ventral surface.

Second maxilliped (*Fig. 3f*). — The exopodite has six long, heavily plumose setae. The endopodite is four-segmented and slightly shorter than the exopodite. The setation formula of the endopodite is 5, 3, 3, 2. The basis has three setae ventrally on the distal half of the segment.

Third maxilliped (*Fig. 3g*). — The exopodite has six plumose terminal setae. The endopodite arises from the midpoint of the side of the basis and extends beyond the end of the basis. The endopodite is not segmented from the basis; terminally it has one long and one short seta.

Pereiopods (*Fig. 3h*). — The pereiopods are larger relative to the rest of the body than in the previous stages, but segmentation is not visible. The cheliped is now the most prominent of these appendages, and the dactylus and its propodal extension, the opposed spine, are apparent.

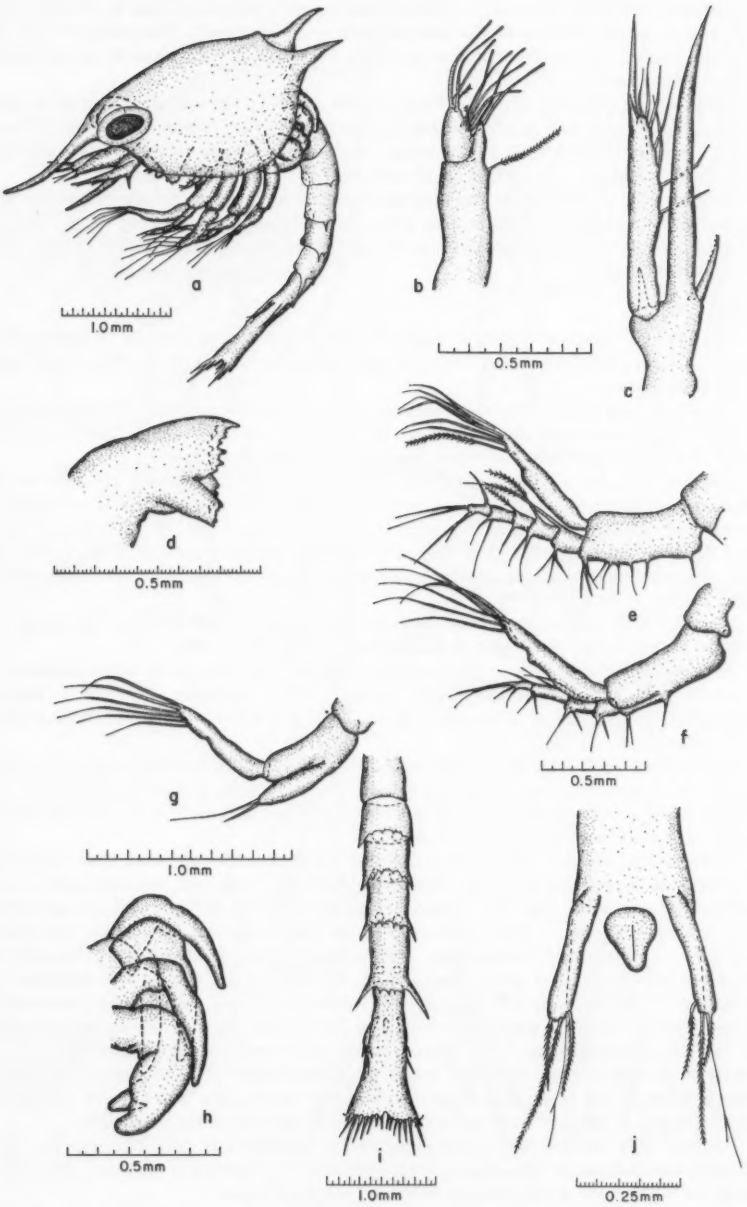

FIG. 3. (*a*) Stage III zoea of *P. platypus*; (*b*) antennule; (*c*) antenna; (*d*) mandible; (*e*) first maxilliped; (*f*) second maxilliped; (*g*) third maxilliped; (*h*) pereiopods; (*i*) abdomen and telson; (*j*) uropods.

Abdomen and telson (*Fig. 3i*). — The abdomen and telson are similar to those of stages I and II except for the addition of the pleopod buds and the uropods. The pleopod buds are on the postero-ventral region of abdominal segments 2 through 5. The uropods are on the telson lateral to the anterior lip of the anus.

Pleopod buds (*Fig. 3a and 3i*). — Pleopod buds generally show as small bumps on the exoskeleton of the abdomen and as internal tissue buds; the bumps are present in the stage III exuviae. In a few specimens, which may be abnormal, the pleopods appear only as tissue buds beneath the exoskeleton and are not evident on the exoskeleton.

Uropods (*Fig. 3i and 3j*). — The uropods, first present in stage III, are each composed of a single segment. Each bears three plumose setae, two terminal and one subterminal. The middle seta on each uropod is approximately twice as long as the lateral and slightly less than twice as long as the medial seta.

Stage IV Zoea

The average carapace length of stage IV zoeae (Fig. 4*a*) is 2.0 mm (8 specimens); the average total carapace length is 4.5 mm (8 specimens); and the average total body length is 6.8 mm (8 specimens).

Antennule (*Fig. 4b*). — The distal segment of the antennule has eight or nine aesthetes and two setae. The medial extension or lobe of the proximal segment and the distal segment are subequal in length. The medial extension has a plumose seta at its base.

Antenna (*Fig. 4c*). — The antenna is similar in shape, setation, and spination to that of previous stages. The basal segment bears two stout spines. The medial spine is segmented from the basis, and the distal half is denticulate. The lateral spine is smaller, smooth, and not segmented from the basal segment. The exopodite has eight sparsely plumose setae on the medial margin and terminates in a long, slender spine which makes the exopodite and endopodite subequal in length.

Mandible (*Fig. 4d*). — The mandible is similar to that of the previous two zoeal stages. The molar process is well developed. A mandibular palp is not present.

First maxilliped (*Fig. 4e*). — The exopodite has six long, heavily plumose terminal setae. The endopodite is five-segmented and equal in length to the exopodite. The setation formula of the endopodite is 5, 2, 2, 3, 4, as in zoeal stages II and III. The basis has nine small setae on its ventral surface.

Second maxilliped (*Fig. 4f*). — The second maxilliped is the same as in stages II and III. The exopodite has six long, heavily plumose setae. The endopodite is four-segmented and shorter than the exopodite. The setation formula of the endopodite is 5, 3, 3, 2. The basis has three setae located on the distal half of its ventral margin.

Third maxilliped (*Fig. 4g*). — The exopodite has six plumose terminal setae. Some indication appears of segmentation of the endopodite from the basis, but segmentation is not yet complete. The endopodite has three sparsely plumose setae, two terminal and one subterminal.

Pereiopods (*Fig. 4a*). — The relative size of the pereiopods has increased so that they now project below the margin of the carapace. The pereiopods have all segments of the adult stage. None of the pereiopods have setae. The cheliped, pereiopod 1, has two rows of denticles on the inner surface of the dactylus and its propodal extension. Within each of the pereiopods 2 to 5, all segments are about the same diameter except the terminal segment, which is always smaller.

Abdomen and telson (*Fig. 4h*). — The abdomen and telson of stage IV zoeae have spination and setation similar to those of earlier stages. Abdominal segments 2 through 5 bear pleopods. The uropods are on the telson just anterior and lateral to the anus. The anterior portion of the telson of the zoea is destined to be the sixth abdominal segment of the glaucothoe.

Pleopods (*Fig. 4h and 4j*). — The pleopods are composed of two segments and bear no setae. Each pleopod has an extension on the medial side of the proximal segment. This extension becomes the endopodite of the pleopod in the glaucothoeal stage.

Uropods (*Fig. 4h and 4i*). — The uropods are two-segmented: the proximal segment is slightly less than one-half the length of the distal segment. The distal segment of each uropod has three plumose setae. Both the lateral and the medial setae are about two-thirds the length of the median seta.

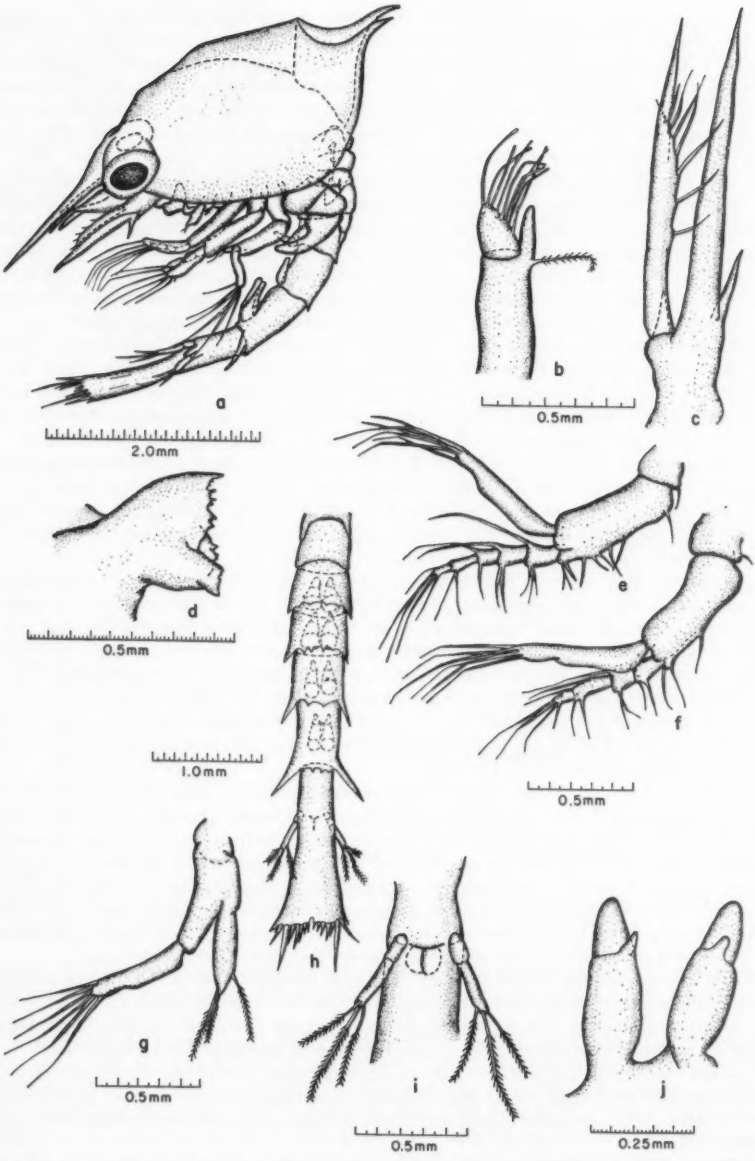

FIG. 4. (*a*) Stage IV zoea of *P. platypus*; (*b*) antennule; (*c*) antenna; (*d*) mandible; (*e*) first maxilliped; (*f*) second maxilliped; (*g*) third maxilliped; (*h*) abdomen and telson; (*i*) uropods; (*j*) pleopods of fifth abdominal segment.

GLAUCOTHOE

The glaucothoes (Fig. 5 and 6) have an average carapace length of 1.8 mm (6 specimens), 0.2 mm less than the comparable measurement of stage IV zoeae. A decrease length with the change in body form from zoea to glaucothoe is usual in larvae of *Paralithodes*. The average total carapace length is 2.6 mm (six specimens). The average width of the carapace of the glaucothoes is 1.7 mm (six specimens). The total lengths of the glaucothoes were not determined because death causes the abdomen to be flexed so tightly against the ventral surface of the body that accurate measurements cannot be made.

The carapace (Fig. 5*a* and 6*a*) of the glaucothoe has 15 pairs of spines on its dorsal surface, not including those of the rostral complex (frontal area of Makarov, 1938). This complex is discussed separately. In addition to the dorsal spines, the antero-ventral margin of the carapace has a pair of suborbital spines. Each of the spines on the carapace has a small seta on its base. The number of paired spines on the carapace of the glaucothoe was constant on all specimens examined, although their location was slightly variable. The carapace spination is described according to the carapace areas in crab-like anomurans designated by Makarov (1938). The 15 pairs of dorsal spines are disposed as follows: two pairs in the gastric area and two pairs in the cardiac area disposed in two parallel lines from anterior to posterior; one pair in the orbital area; two pairs in the hepatic area; and eight pairs in the branchial areas. Four of the pairs of spines in the hepatic and branchial areas are on the lateral margins of the carapace; the most anterior pair is in the hepatic areas. and the other three pairs are in the branchial areas.

The rostral complex of the carapace (Fig. 6*b* and 6*c*) includes two antero-lateral prominences or spines, each of which has a smaller, medio-dorsally directed spine. Each of these four spines has a small seta on its base. The rostral spine is well developed and is about equal in length to the truncate base of the complex from which it originates. The eyes each have four spines on their anterior surface, and each of these spines has a small seta.

Antennule (*Fig. 5b*). — The antennule of the glaucothoe is composed of a three-segmented peduncle which terminally has a pair of segmented rami. The larger ramus is four-segmented. The distal segment has three terminal setae and three lateral aesthetes, and the next two more proximal segments have at least six aesthetes each. The basal segment of the larger ramus has neither setae nor aesthetes. The smaller ramus of the antennule is two-segmented. The distal segment has three large terminal setae and six small setae, and the proximal segment has two setae. The peduncle has two small setae (neither of which is plumose), one on each of its two most distal segments. The outermost seta of the peduncle is in the position of the large plumose seta of the antennular peduncle of the zoea.

Antenna (*Fig. 5c*). — The antenna of the glaucothoe differs considerably from that of the zoea. The endopodite is now an elongate, nine-segmented flagellum rather than unsegmented as in the zoea. This flagellum has setae on all segments except the third from the basis. The exopodite (antennal scale) is smaller than in the zoea and has one simple seta. The prominent spines that were on the basis in earlier zoeal stages are also smaller, particularly the one below the flagellum.

Mandible (*Fig. 5d*). — The mandible of the glaucothoe has a well-developed, two-segmented mandibular palp which has no setae. This stage is the first in which the mandibular palp is present. The denticles on the mandible are relatively smaller than those of the zoeal stage.

First maxilliped (*Fig. 5e*). — The first maxilliped is smaller than it is in the zoeae. The two-segmented exopodite has five short plumose setae. The two-segment endopodite has four short terminal setae. The exopodite and endopodite both appear shriveled, and their segmentation is difficult to determine because of folds in the exoskeleton. The basis has six small setae.

Second maxilliped (*Fig. 5f*). — The second maxilliped is relatively smaller in the glaucothoeal stage than in the zoeal stages. The exopodite is two-segmented and has five sparsely plumose terminal setae. The endopodite is four-segmented and has one short terminal seta. The basis has no setae.

Third maxilliped (*Fig. 5g*). — The third maxilliped as a whole is relatively larger than in the zoeal stages; but the two-segmented exopodite, which has five terminal setae, is relatively smaller. The endopodite is larger and more highly developed than in previous stages and is seg-

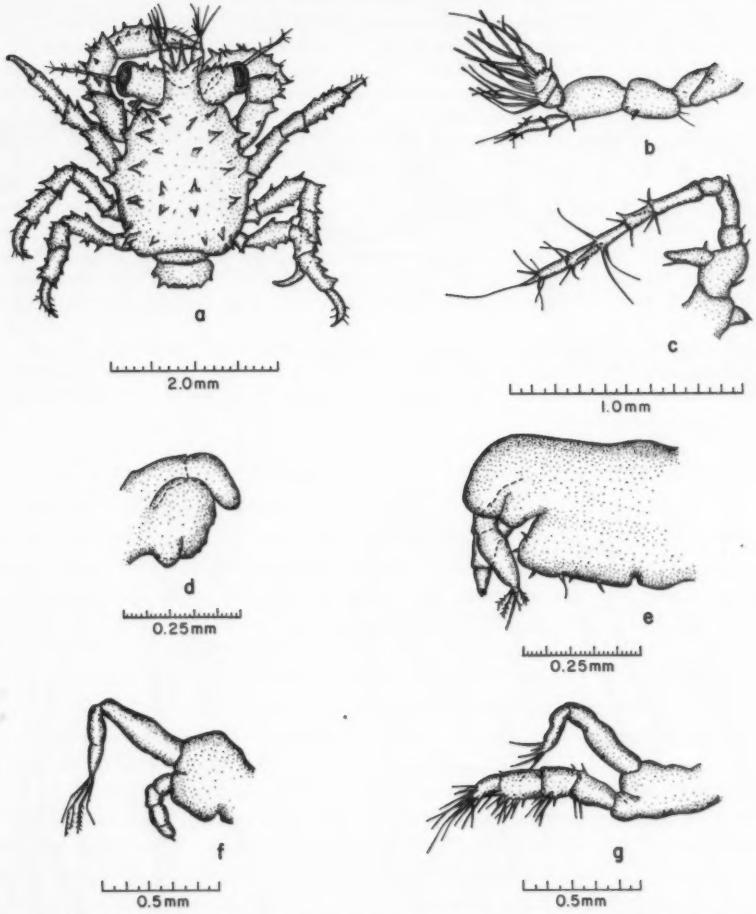

FIG. 5. (*a*) Glaucothoe of *P. platypus*; (*b*) antennule; (*c*) antenna; (*d*) mandible (right); (*e*) first maxilliped; (*f*) second maxilliped; (*g*) third maxilliped.

mented for the first time. The endopodite is composed of four segments, each with robust denticulate setae. The setation formula of the endopodite is 10–14, 10–14, 7, 4, and the setae are concentrated on the ventral surface.

Pereiopods. — The pereiopods closely resemble those of the adult. Pereiopods 1–4 (Fig. 5*a*) bear spines on all segments except the dactyls. The dactyls of pereiopods 1–4 are setose. The right cheliped is larger than the left, but has a similar form. The fifth pereiopod (Fig. 6*d*) is held beneath the carapace in this stage as in post-larval stages. The fifth pereiopod is six-segmented and has setae on the distal three segments; it also has two short simple setae on each dactyl, three long terminally curved setae and six shorter setae on each propodus, and a single seta on each carpus.

Abdomen and telson (*Fig. 6e*). — The segmentation of the abdomen and the spination of the abdomen and telson differ those of zoeal stages. The abdomen is now six-segmented, and

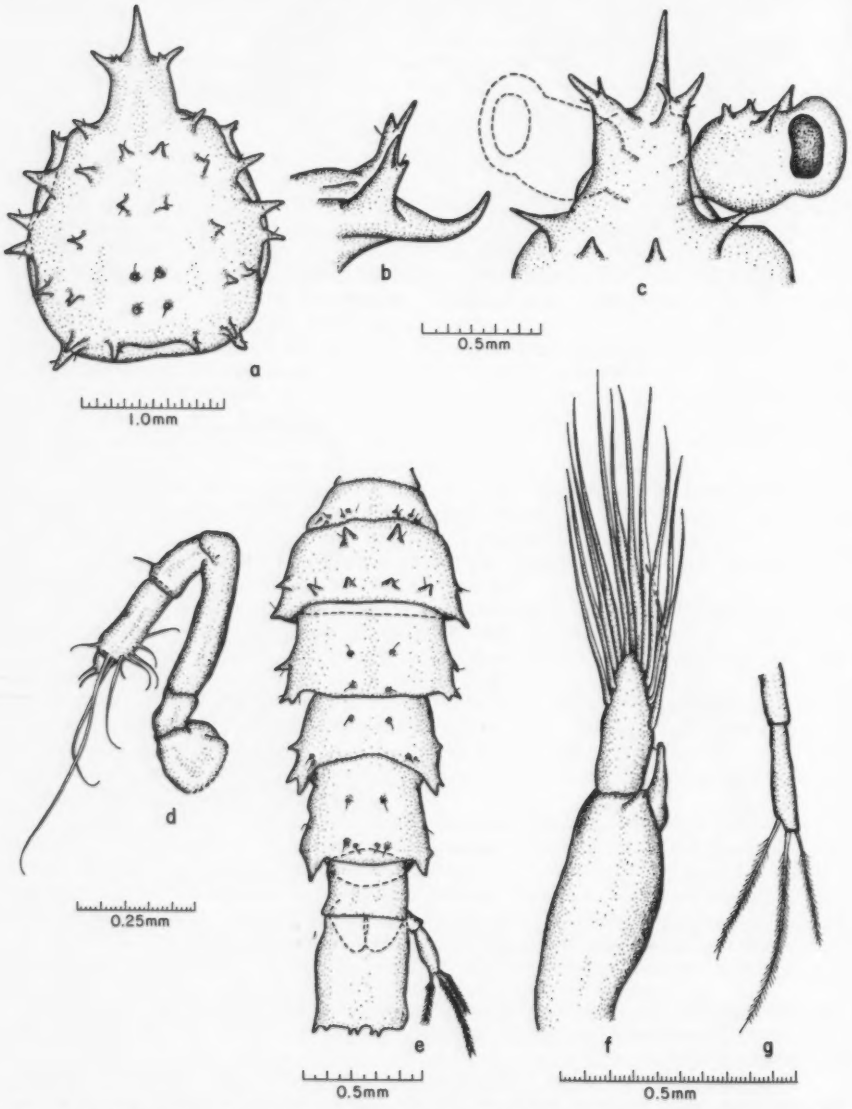

FIG. 6. (*a*) Carapace of glaucothoe of *P. platypus*; (*b*) rostrum; (*c*) eye and rostrum complex; (*d*) fifth pereiopod; (*e*) abdomen and telson; (*f*) pleopod; (*g*) uropod.

the uropods arise from the sixth segment. All abdominal segments have hair-like setae on the dorsal surface. On the second segment these setae occur at the bases of the three pairs of dorsal spines. The spines on this segment are located as follows: one on each side of the midline in the antero-dorsal region and two on each side of the midline in the postero-dorsal region. Each lateral

margin of the second segment has two spines, one somewhat posterior to the middle of the segment and the other on the postero-lateral corner. The postero-lateral corners of segments 3, 4, and 5 are prolonged into backward pointing spines which are bifid in segments 3 and 4. The postero-lateral corners of segments 1 and 6 do not have spines. The setation of the telson varies, but setae are fewer and smaller than in the zoea. The posterior margin of the telson has as few as two pairs or as many as five pairs of setae. A consistent feature of the telson in all larval stages is a seta-free median notch in the posterior margin. This notch is broader and shallower in the glaucothoe than in the zoea.

Pleopods (*Fig. 6f*). — Pleopods are present on abdominal segments 2 through 5, as in zoeal stages III and IV, but are more highly developed. The exopodites of the pleopods 2 through 5, respectively, have 12, 12, 13, and 11 large plumose terminal setae which are not present on the zoeal pleopods. The endopodite, now segmented from the proximal segment of each pleopod, consists of a single segment with three short, stout, hooked setae and one simple seta on the distal end.

Uropods (*Fig. 6e and 6g*). — The uropods are similar to those of stage IV zoca. They are two-segmented; the distal segment of each has three long plumose setae. The lateral seta is slightly more than two-thirds, and the distal seta slightly less than two-thirds, as long as the medial seta.

COMPARISON OF DESCRIPTIONS OF LARVAE OF *P. PLATYPUS*

Rearing the larvae of *P. platypus* in the laboratory permitted the verification or correction of some earlier descriptions of the zoea. My materials also provided a basis for more description of the development of the zoea and the first description of the glaucothoe. The development of the larvae of *P. platypus* is similar to that of other species of the family Lithodidae. Gurney (1942), Pike and Williamson (1959), MacDonald et al. (1957), and Hart (1965) discussed similarities of larval development among the species of Lithodidae. My discussion is limited therefore to the differences among the various descriptions of the larval development of *P. platypus* and some similarities within the genus *Paralithodes*.

The larvae described in this study differ in several ways from the descriptions of Marukawa, Sato, and Kurata. The differences may arise partly from the sources of the specimens described. Marukawa (1933) used stage I zoea whose origin is unknown to me. The first zoeae used by Kurata (1964) were from the plankton. Sato (1958) obtained zoeae of stages II–IV from the intestinal tracts of juvenile pink salmon.

Marukawa (1933) presented a figure of the whole stage I zoeae of *P. platypus* but illustrated in detail only the telson and antenna. The arrangement of telson processes that I found are like those shown in Marukawa's figure except that his figure lacked the 2nd hair-like telson process. Marukawa did not describe the appendages of *P. platypus* in his text. The antenna was illustrated by Marukawa with two well-developed spines on its basal segment, but the proximal one lacked the denticulations that I found. Points of agreement between Marukawa's description and mine are the eight setae on the medial margin of the antennal scale and the subequal lengths of the exopodite (antennal scale) and the endopodite. The tip of the endopodite in Marukawa's figure is simple, rather than bifid as I have found. This difference may be a matter of individual variation rather than a specific character. The average

total body length of stage I zoeae in the present study (4.88 mm) is similar to that found by Marukawa (4.98 mm). Carapace lengths cannot be compared because the points from which Marukawa's measurents were made are not stated.

Kurata (1964) described only the stage I zoea of *P. platypus*. He indicated that this stage had 9 + 9 telson processes. I found these numbers to apply in all the zoeal stages. The lateral spines of the fifth abdominal segment of my stage I zoeae were longer than those described by Kurata. He stated that they reach approximately three-fourths of the distance to the anterior lip of the anus, but I found that although these spines were variable, they generally extended to the level of the anterior lip of the anus (all zoeal stages). Kurata found nine setae on the medial margin of the antennal scale, whereas I found eight.

Sato (1958) did not describe in detail the zoeal stages of his specimens (from the intestinal tracts of juvenile pink salmon); only his illustrations are available, therefore, for comparison with my larvae.

The most striking difference between Sato's (1958) zoeal material and mine is the larger size of the lateral spines of abdominal segments 3 and 4 in my specimens. The lateral spination of the abdominal segments of *P. platypus* illustrated by Sato resembles that of *P. camtschatica* more than that of my specimens. The sixth abdominal segment is separated from the telson in zoeal stages III and IV in Sato's figures, but I did not find this separation until the glaucothoeal stage.

I found the following differences between the setation of Sato's (1958) figures and mine: Sato showed nine setae on the antennal scale instead of the eight I consistently found in all zoeae. Sato illustrated the exopodites of maxillipeds 1–3 in stage II zoeae with 7, 7, and 6 setae and all maxillipeds of stages III and IV with eight plumose setae on each of the exopodites. In contrast, I found 4, 4, and 0 plumose setae on exopodites of maxillipeds 1, 2, and 3, respectively, in zoeae of stage I, and six plumose setae on the exopodite of maxillipeds 1, 2, and 3 in all other zoeal stages. The uropods of the stage III zoeae illustrated by Sato have three short spines rather than the three long slender plumose setae I found.

One of the few morphological features present in all of the developmental stages of the three species of *Paralithodes* whose larval development has been studied is the medial notch of the posterior margin of the telson. This notch was once thought to be characteristic of all species of the family Lithodidae, but larvae of *Cryptolithodes typicus* do not have it (Hart, 1965).

In the genus *Paralithodes*, some species have three and others four zoeal stages. The uropods of these zoeae are always uniramous and have three setae. The uropods of the last zoeal stage always have two segments. They have one segment in stage III in those species with four zoeal stages. Species with three zoeal stages lack uropods in stage II.

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The Relationship of Midwater Trawl Catches to Sound Scattering Layers off the Coast of Northern British Columbia[1]

By F. H. C. Taylor

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

ABSTRACT

In 1964 and 1965 three midwater trawl cruises off the continental slope near the Queen Charlotte Islands were made to study the relationship of the catches to sound scattering layers.

During the daytime four layers were distinguished, not all necessarily present at one time. In summer the most intense and persistent layer lay at 220–230 m; others were at 330–400, 185, and 90 m. In fall the main and deep layers were shallower. Vertical migration occurred at dusk and dawn. The main layer frequently split into three parts with different vertical migration patterns. At dawn a layer gradually appeared at about 90 m, intensified rapidly, and descended to the daytime level.

Catches show fishes are associated with the sound scattering layers. The largest day catches were from the main layer. At night fish did not appear to be concentrated to the same extent relative to the layers. The largest night catches were usually smaller than the largest day catches and shallower.

Myctophids of eight species, four common, made up 80% of the catch, chauliodontids 6%, argentinids 4%, melamphids, rockfish, and larval flatfish 2% each. The remaining 2% comprised 27 species in 21 families. Depth distribution and vertical migration varied considerably with the species.

Myctophids with gas-filled swim bladders were apparently dominant in the main layers whereas species without swim bladders — stomiatoids, argentinids, and melamphids — or myctophids with fat-filled swim bladders were dominant below the main layer and in the deep layer. Fish with no swim bladders or with fat-filled ones may make longer vertical migrations than those with gas-filled bladders.

INTRODUCTION

Deep sound-scattering layers in the ocean were first recognized in 1942 (Duvall and Christensen, 1946; Eyring et al., 1948; Raitt, 1948). They have since been found to be widespread in most of the major oceans except the Arctic and Antarctic oceans (Dietz, 1948; Hersey and Moore, 1948; Tchernia, 1950; Tucker, 1951). Recently a persistent but relatively shallow scattering layer (50–200 m) has been reported in summer beneath Fletcher's Ice Island (T3) in the central Arctic Ocean (Hunkins, 1965).

Johnson (1948) described the diurnal movements of these layers and was the first to try to link them with concentrations of plankton. He concluded the correlation "may be partly secondary, since the more effective scatterers may be larger forms that subsist upon and migrate more or less concurrently with the plankton." The association of plankton concentrations,

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principally of euphausiids, with the scattering layers has been demonstrated by Johnson (1948), Moore (1950), Kampa and Boden (1954), and Barham (1957). However, whether this relationship is a primary or secondary one remains an open question.

Most observations on the scattering layers have been made with single frequency echosounders operating from the surface. If a broad spectrum sound source is used several layers may be detected in one locality, each having a different peak-scattering frequency (Hersey et al., 1952; Johnson et al., 1956; Kampa and Boden, 1954). The concept of there being horizontal homogeneous layers of scatterers may be erroneous (Barham, 1957). A complex dynamic situation may exist, of which different segments are illuminated at different frequencies. Furthermore, as some layers migrate in depth there is a gradual shift in their peak-scattering frequency (Hersey and Backus, 1954; Hersey et al., 1961). Johnson et al. (1956) interpret this as meaning that "the scatterers change in size (resonant frequency) with changing depth (pressure) such as might be expected if the scatterers were [gas-filled] swim bladders of fishes." This conclusion supports the contention that bathypelagic fishes are the chief cause of deep scattering layers (Marshall, 1951, 1960). Tucker (1951) has shown that there is a correlation between deeper, more intense scattering and the vertical distribution of fishes, and the shallower less intense scattering and the distribution of euphausiids.

Using an echosounder suspended in the scattering layer and an attached camera, Johnson et al. (1956) demonstrated that images of fish on photographs were always accompanied by strong echo sequences, but strong echo sequences were not always accompanied by fish images. At least in part this is because the fields of view of camera and sounder were not identical. Similar results were obtained by Backus and Barnes (1957) using a television camera and attached sounder lowered into the scattering layer.

Although there is thus a considerable body of evidence mainly from sound studies, in some case coupled with photography or television, to show that bathypelagic fish are associated with the deep scattering layer and in many instances may be a major cause, there have been few attempts to relate catches of fish and sound layers.

In 1964 and 1965 a study was carried out to determine whether the fish associated with the sound scattering layers (as portrayed at frequencies of 30 and 38.5 kc) off the rich commercial fishing grounds in Queen Charlotte Sound were sufficiently numerous to form an exploitable resource in their own right, whether they formed an important source of food for commercially exploited species, and whether among the species represented there were the young of commercial species. In this report the data obtained are used to provide information on the relationship between the size and composition of the catch and the various scattering layers encountered. Detailed catch records by haul, and descriptions of the more uncommon species taken, are given elsewhere (Taylor, 1967a, b).

METHODS

Collections of bathypelagic fishes were made on three cruises of the research vessel *G. B. Reed* in June 1964 (Cruise 64-6), October–November 1964 (Cruise 64-12), and July 1965 (Cruise 65-7) to the general area beyond the continental shelf off Queen Charlotte Sound and the southern part of Moresby Island in the Queen Charlotte Islands group (Fig. 1). Water depths varied from about 915 to 2750 m.

The nets used were large German Engel trawls developed for herring midwater trawling (Schärfe, 1960). On the first and third cruises the net used was 1200 20-cm meshes (stretched measure) in circumference; mesh size graded down to 2 cm in the codend. The headline measured about 38 m and the breastlines about 35 m. On the second cruise a slightly smaller net constructed in exactly the same way was used. The only major differences were that the 50-mesh long strip of 20-cm mesh was omitted from the front of the body and the wings were of 16-cm mesh instead of 20-cm mesh. The net was thus 1200 16-cm meshes in circumference. With both nets a vertical mouth opening of 13–15 m was obtained when fishing. Süberkrüb otter-boards of 5 m^2 (Schärfe, 1960) were used to spread the net. On the first cruise 150-m bridles were used; on the second and third cruises 55-m bridles were found more satisfactory. Two 300-kg weights were attached to the lower wing corners. The headline was buoyed with 82 8-inch (20.3 cm) Phillips aluminum floats.

The effective area of the mouth of the net is difficult to determine because of problems associated with the different selectivity of the various mesh sizes. It is thus impossible to express the catch quantitatively in terms of numbers of individuals per volume of water strained. However, catches are probably comparable with one another, provided adjustments are made for differences in towing time and speed. The actual catches have therefore been expressed as catches per hour at an average speed of 2.8 knots (5.2 km/hr). The somewhat smaller circumference of the net used for the small number of tows on the second cruise may affect the comparability of the catches made but probably not significantly since both nets had the same sized effective vertical mouth opening and since the body mesh sizes in both were large compared with the size of the fish caught. The number of fish observed entangled in the body meshes suggest that the relatively weak swimming bathypelagic fish are probably not guided into the net to any appreciable extent by large mesh netting but may be washed through it.

Some contamination of the catches of deeper hauls may have occurred with the passage of the net through shallower layers on setting and hauling. However, the amount is probably small as the length of time the net would spend in the shallower concentration was short in comparison to towing time at the desired depth.

The *G. B. Reed* is equipped with two Simrad 510-5 echosounders, operating on 38.5 kc and 53.0 kc, and a 30-kc Simrad 580-5 echoranging set which can also be used as a sounder. The 510-5 sounders had a pulse power output of 450 w; the 580-5 echoranger of 1000 w. The sounders have four basic

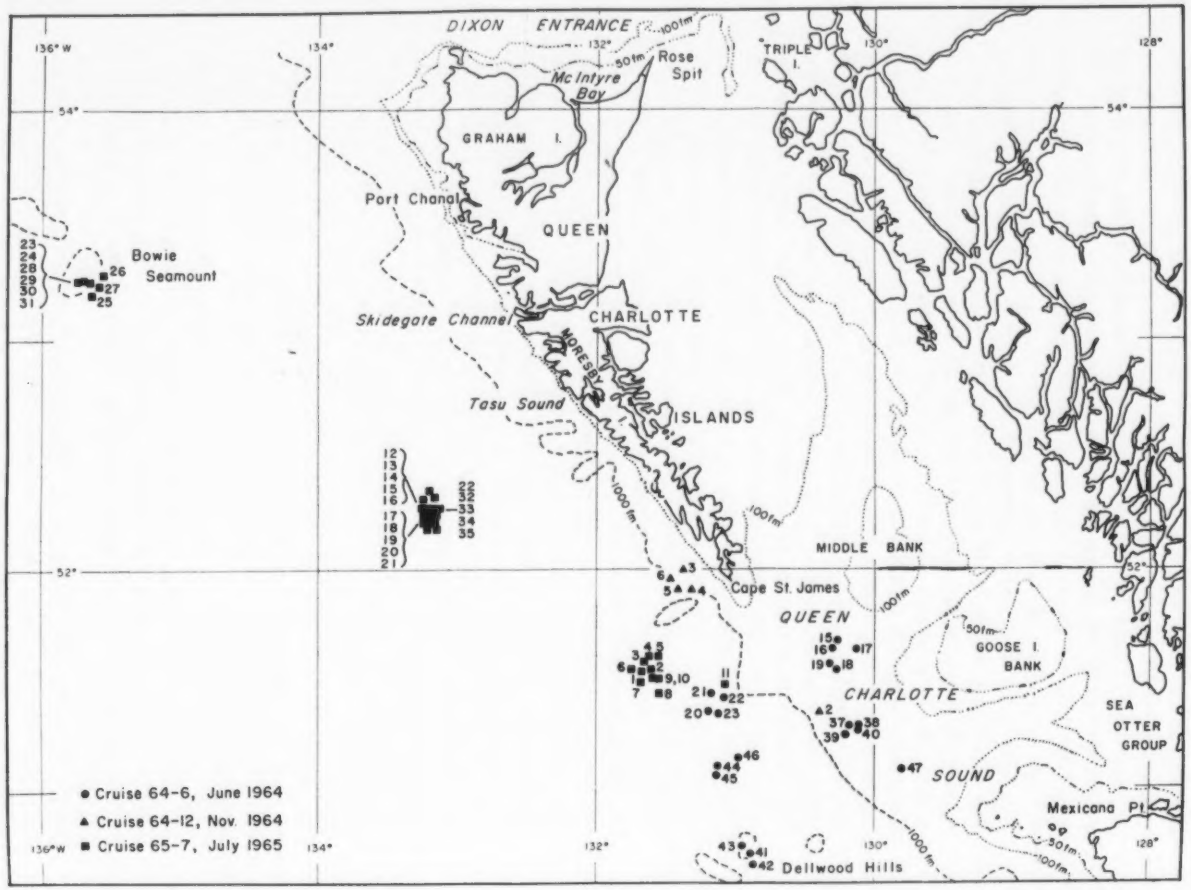

FIG. 1. Northern and central British Columbia coast, showing the location of hauls made beyond the continental shelf.

range scales of 0–70, 0–140, 0–275, and 0–550 fath,[2] each with four phasing steps overlapping by about 25%. The echoranger has two basic ranges: 0–800 and 0–135 fath, the latter with two phasing steps of 100–235, and 200–335 fath.[2]

An 11-kc Edo Model 185 sounder with a Westrex precision depth recorder was available but not used as the sounder was operationally unreliable.

A 10 × 16-cm transducer operating on 38.5 kc was fastened to the net and used to monitor its depth. This transducer was operated through about 730 m of Amergraph type 3-H-1 double armoured cable. Depth gauges, providing a record of depth against time on a small circular chart, were used on some hauls. The net transducer method was preferred because of the greater amount and greater accuracy of the information provided. Not only was the depth of the net evident at all times but also its relation to the target layer was immediately apparent. Adjustment could be made to the depth of the net while fishing to ensure it retained the desired relation to the target. The ratio of towing warp to depth used was about 4:1. Small adjustments in net depth were made by adjusting vessel speed. By this method the net could readily be maintained within 3–9 m of the desired depth.

SCATTERING LAYER OBSERVATIONS

Observations on the depth and movement of the sound scattering layers were made using the Simrad 580-5 echoranging set as a sounder rather than the less powerful Simrad 510-5 sounders. The latter provided additional more detailed information at shallow depths. During the day four scattering layers were distinguished, but not all were necessarily present at the same time.

June and July

The main or third scattering layer, the most intense and persistent, lay generally between 220 and 330 m from the surface, usually at about 275 m in daytime. Below this a deeper layer, the fourth, was often observed at about 330–400 m. This layer was generally faint and rather intermittent. Above the main layer, one and sometimes two shallower layers were present. The second layer was generally centered about 185 m but varied from 145 to 220 m. This layer was generally, but not always, present. It was considerably less intense than the main layer. The first layer was the least intense and the most intermittent. When present, it was usually found at about 90 m but varied between 75 and 130 m. The relatively few observations made in October–November suggested the main layer was generally a little shallower, lying at about 185 m, and the deep layer possibly also somewhat shallower, lying between 310 and 365 m, but considerably more intense than in summer. The two upper layers were not observed at this season in the daytime.

[2]The equivalent model of echosounder with metric scales has basic ranges of 0–125, 0–250, 0–500, and 0–1000 m, each with four phasing steps. The equivalent model of echoranger has two basic ranges: 0–1500 and 0–250 m, the latter with two phasing steps of 200–450 and 450–650 m.

FIG. 1. Northern and central British Columbia coast, showing the location of hauls made beyond the continental shelf.

In the evening the layers rose towards the surface, descending again towards dawn. Rates of ascent and descent of the order of 55–90 m per hour were observed.

The ascent appeared to have two phases. First there was a slow upwards drift, primarily of the main and second layers. This occurred between about 1800 and 2100 hr (PST) and covered 35–90 m. This upwards drift was followed by a rapid rise of 140–230 m by parts of the main layer (Fig. 2). This ascent usually began between 2100 and 2200 hr and was completed by 2300 to 2330 hr. The main layer seemed to split into three sections. One part tended to remain at the daytime level of about 220–275 m or to rise only slightly. The second part rose to 140–185 m, merging with and intensifying the second layer. The third part continued the rapid ascent to within 45–90 m of the surface, sometimes merging with the upper (first) layer or with the surface layer. Both this part of the main layer and the upper layer tended to fade out shortly after the main ascent. By midnight there were thus usually two layers present, one, the most intense, at 140–185 m, formed probably of parts of the main layer and of the second layer, and the other about 220–275 m (off the recording in Fig. 2*h*), being that part of the main layer which had not migrated. Sometimes the first layer persisted after the evening ascent (Fig. 2*g* and 2*h*).

Few observations on the behaviour of the deep layer were made. In one instance an upwards drift of 45 m was observed about 1700 hr, after which the layer faded out. However, there sometimes appeared to be indications of an ascent towards the main layer at between 1900 and 2000 hr, or between 2200 hr and midnight. There were also sometimes faint indications of an ascent from the deep layer to the next layer above it between 0200 and 0400 hr.

The main descent occurred very rapidly over about 1.5–2 hr, between approximately 0400 and 0600 hr (Fig. 3). The layer gradually appeared at about 90 m over a period of possibly an hour. This was followed by a very pronounced intensification and rapid descent of this layer and that part of the main nighttime layer at 140–185 m to approximately the daytime level of 220–275 m. Part of the layer apparently remains at the 185-m level forming the daytime second layer. A slow descent covering about 45 m occurred throughout the morning so that the layers reached their deepest point between about noon and 1400 hr. In two or three instances a faint layer was seen to break away from the lower nighttime layer (at about 230–275 m) and to descend and fade out.[3] This may be part of the daytime deep layer descending.

The movements of the second layer are not as clearly demonstrable from the observations available as those of the first layer. Movement upwards and downwards appears to be synchronized with that of the main layer. Sometimes early in the evening the second layer splits, part drifting upwards and fading out (Fig. 2*a–c*). Part remained at about its original level. This part

[3]Signal strength decreases as the square of the distance between transducer and target. As a layer producing a weak signal descends, signal strength will decrease rapidly, so the layer may fade out for this reason. It is also possible that as the layer descends it disperses until it is no longer dense enough to return an echo.

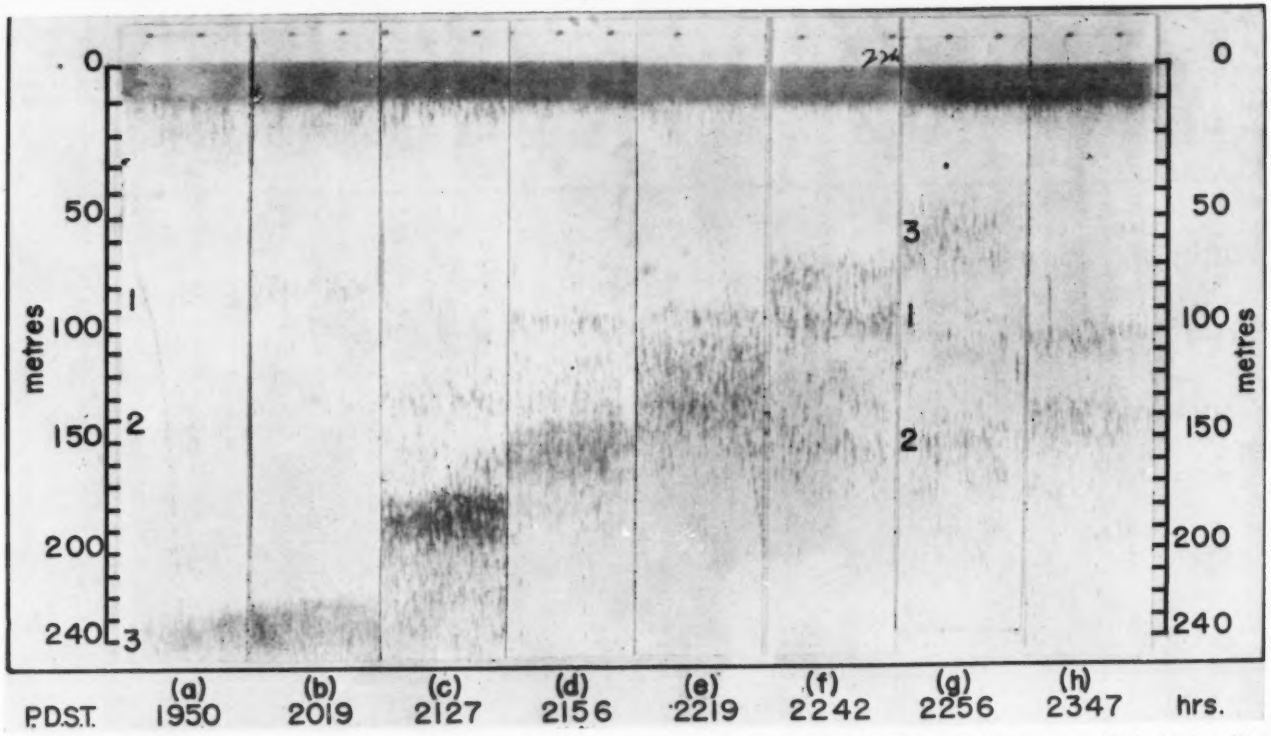

FIG. 2. Ascent of scattering layers, July 9, 1965. The third layer (3) rose from 220 m (*a*), crossing the second (2) at 128 m (*d*, *e*), the first (1) at 90 m (*c*, *f*), and fading out at 55 m (*h*). The first and second layers maintained approximately their original positions. The second layer split (*a*, *b*), part rising to 110 m before fading out. Intensity of the third layer decreased and of the first and second increased.

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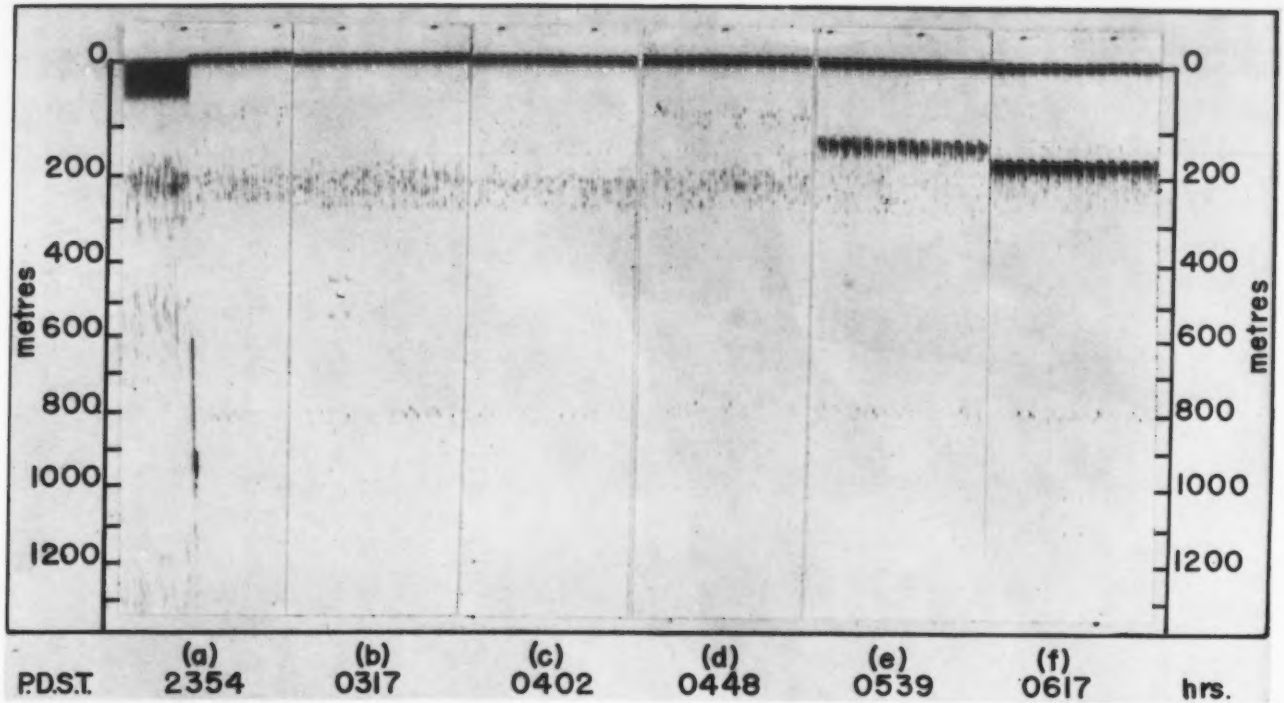

FIG. 3. Descent of scattering layers, July 11, 1965. Part of the third layer remained at about 220 m throughout the night (*a–e*). The migratory part faded in at about 90 m (*d*), intensified and descended rapidly (*e, f*). The left half of (*a*) spans the range 0–250 m, this being the same scale as used in Fig. 2.

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became more intense after the main ascent (Fig. 2*f–h*). After the main descent a weaker layer remained behind at the original level of the second layer. Part of this second layer could have been carried up or down by the movement of the main layer, but part was obviously not affected.

During the day the depth of the main layer oscillated somewhat, varying up to 35–45 m in 2–3 hr. The less intense first and second layers moved as well, often in synchrony, at times splitting and merging with each other. The daytime depth variations would be a result of vertical movement or of changes in the depths of the layers associated with horizontal distribution.

November

The layers exhibited the same general pattern of diel migration as in summer, but because of the shallower general depth of the layer the ascent and descent covered a somewhat shorter distance. The time of ascent varied from about the same to an hour later.

Horizontal Distribution

The scattering layers continued in to the continental slope (from 200 to 1800 m), which acted as a boundary. At the edge of the slope the layers lay slightly above their usual open ocean depth. In some instances the deep layer appeared to bend up and merge with the main layer just before reaching the slope. On the edge of the slope, lying above the main layer, numbers of separate cloudlike schools were sometimes found.

CATCHES

A total of 49 midwater trawl hauls (Fig. 1) were made in various positions relative to the sound scattering layers present in waters beyond the continental shelf. Of these hauls, 20 were made on Cruise 64-6, 14 by day and 6 at night; 5 on Cruise 64-12, 2 by day and 3 at night; 24 on Cruise 65-7, 17 by day and 7 at night. Table 1 shows the depth of each haul, its position with reference to the sound scattering layers and the catch per hour. Because of the complex layer structure encountered the number of hauls per cruise in any one position relative to the layers was unfortunately small. Where repeated hauls were made in one position, the small amount of data available suggests the between-haul variability in catch was at times quite large.

Abundance Relative to Depth and Scattering Layers

Catches from deep hauls.— Two hauls were made to below the deepest scattering layers recorded: one on Cruise 64-6 to about 730 m, the other on Cruise 65-7 to 510–595 m. The catches from these deep tows, among the largest from each of those cruises, differed considerably from the other catches in species composition. Myctophids were the dominant group in all catches beyond the continental shelf. In the two deep tows argentinids and melamphids were also numerous. Of the myctophids, *Lampanyctus leucopsarus* was the

TABLE I. The catch per hour[a] of the more frequently caught species (or families) for each haul in relation to sound scattering layers and depth.

Position re sound scattering layer	Depth range (*m*)	Cruise and haul no.	Day or night	*Argentinid* sp.	*Tactostoma macropus*	*Chauliodus macouni*	*Hierops thompsoni*	*Diaphus theta*	*Tarletonbeania crenularis*	*T. taylori*	*Tarletonbeania* sp.	*Lampanyctus leucopsarus*	*L. ritteri*	*L. regalis*	*L. nannochir*[d]	*Atheresthes stomias*	*Sebastodes alutus*	*Sebastodes* sp.	Other species
In surface layer	0–45	64-6-38	D	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	0–45	64-6-43	D	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+
Above main layer	90–140	64-6-15	D	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	140–185	64-6-42	D	–	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–
	140–185	64-6-45	D	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	6
In main layer	140–185	64-6-17	D	–	–	–	–	–	–	–	–	1	–	–	–	10L[b]	3	–	1
	140–185	64-6-16	D	–	–	–	4	–	–	–	–	–	–	–	–	37L[b]	9	1	1
	185–230	64-6-39	D	–	–	3	8	6	–	–	–	50	–	–	–	–	18	3	3
	185–230	64-6-41	D	–	–	11	175	179	24	–	–	38	–	–	–	–	–	–	1
	185–230	64-6-47	D	–	–	6	10	20	1	–	–	9	–	–	–	–	6	–	16
	230–275	64-6-37	D	–	–	1	31	139	6	–	–	14	–	–	–	–	5	–	12
	230–275	64-6-44	D	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
	230–275	64-6-20	D	–	–	13	39	4	–	–	–	52	–	–	–	–	–	–	1
Below deep layer	640–730	64-6-21	D	67	18	34	6	41	6	–	–	131	–	77	–	–	–	2	112
Above main layer	90–140	64-12-4	D	–	5	–	4	16	3	–	–	1	–	–	–	–	–	–	1
In main layer	185–230	64-12-3	D	–	–	–	13	175	4	–	1	–	–	–	–	–	–	–	–
Above first layer	45–90	65-7-1	D	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2J[c]	2
	45–90	65-7-23	D	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2
In first layer	45–90	65-7-15	D	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2J
In second layer	90–140	65-7-4	D	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2
	140–185	65-7-3	D	4	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–
	140–185	65-7-9	D	–	–	–	–	–	–	–	–	–	–	–	–	–	–	15J	–

Above main layer	140–185	65-7-14	D	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	185–230	65-7-8	D	–	–	2	–	–	2	–	–	–	–	–	–	–	–	31J	2
	230–275	65-7-2	D	–	–	–	293	19	–	4	12	24	–	–	–	–	–	19J	2
In main layer	230–275	65-7-7	D	–	–	77	724	493	11	–	11	118	–	–	–	–	–	19J	–
	230–275	65-7-13	D	–	–	66	652	433	11	–	5	252	3	–	–	–	–	5J+3	8
	275–320	65-7-27	D	–	–	54	297	29	20	–	–	2	–	–	–	–	–	–	–
	320–365	65-7-12	D	–	–	29	91	930	87	–	24	18	3	–	2	–	–	1J	4
Below main layer	320–365	65-7-6	D	27	–	49	75	725	37	17	12	32	–	–	–	–	–	34J+2	1
	365–410	65-7-18	D	30	2	32	21	93	–	–	–	21	17	–	–	–	–	2	4
	410–455	65-7-26	D	2	–	242	65	121	24	–	–	93	–	–	–	–	–	2	2
In deep layer	410–455	65-7-20	D	8	13	42	4	–	–	–	2	10	–	–	–	–	–	2J+2	19
Below deep layer	510–595	65-7-19	D	217	26	78	5	8	–	–	1	1026	1	17	40	–	–	2	24
In surface layer	0–45	64-6-19	N	–	–	–	–	–	–	–	–	–	–	–	–	162L	–	–	3
	0–45	64-6-23	N	–	–	17	2	14	–	–	–	–	–	–	–	–	–	–	–
Between first and second layers	45–90	64-6-18	N	–	–	–	–	–	–	–	–	–	–	–	–	58L	–	–	4
In second layer	90–140	64-6-40	N	4	1	6	2	194	2	–	–	104	–	–	–	–	–	–	2
In main layer	140–185	64-6-46	N	–	–	10	17	–	–	–	–	95	–	–	–	–	–	–	–
	185–230	64-6-22	N	–	–	54	38	80	11	–	–	19	–	–	–	–	–	–	–
In surface layer	?	64-12-2	N	14	27	–	–	558	38	–	–	126	–	–	–	–	–	–	44
Above main layer	90–140	64-12-6	N	1	13	6	–	220	23	–	–	84	4	9	–	–	–	–	6
In main layer	140–185	64-12-5	N	1	13	17	–	139	3	–	–	123	23	12	–	–	–	–	1
In second layer	90–140	65-7-5	N	12	–	–	10	184	6	2	4	35	–	2	–	–	–	2J	4
In main layer	90–140	65-7-22	N	9	–	14	9	49	2	–	–	55	–	–	–	–	–	–	2
	140–185	65-7-10	N	–	–	17	78	376	10	–	4	233	–	–	–	–	–	21J	6
	140–185	65-7-16	N	–	–	–	6	83	–	3	3	6	–	–	–	–	–	–	–
	185–230	65-7-21	N	–	–	45	56	76	2	2	–	2	–	–	–	–	–	–	–
Below main layer	230–275	65-7-17	N	40	42	28	132	78	2	–	2	132	25	18	–	–	–	–	20
	275–320	65-7-11	N	24	15	35	137	107	28	–	9	107	–	37	2	–	–	3J+2	26

[a]At a speed of 5.2 km/hr.
[b]L = larval form.
[c]J = juvenile.
[d]*Lampanyctus nannochir* may be synonymous with *L. leucopsarus*.

dominant species in the deep tows as opposed to *Hierops thompsoni* or *Diaphus theta* in the other tows.

Day and night catches.— Although the number of hauls per cruise at any one position relative to the layers is small, it would appear that in the daytime there were more fish in the main layer than above or below it, whereas at night fish were not concentrated to the same extent in any one position relative to the layers. In Cruise 65-7, except for the deep haul, the average total catch of all species per hour of fishing at 5.2 km/hr (Table II) was greatest

TABLE II. The day and night average total catch of individuals per hour of fishing in relation to various scattering layers for each cruise. The number of hauls is shown in parentheses.

	Cruise 64-6 June		Cruise 64-12 October–November		Cruise 65-7 July	
	Day	Night	Day	Night	Day	Night
Surface layer	+[a](2)	99(2)	–[b]	807(1)	–	–
Above first layer	–	–	–	–	3(2)	–
Between first and second layers	–	62(1)	–	–	–	–
In second layer	–	315(1)	–	–	7(3)	261(1)
Above main (third) layer	3(3)	–	30(1)	366(1)	137(3)	–
In main layer	123(8)	162(2)	193(1)	332(1)	1120(4)	292(4)
Below main layer	–	–	–	–	595(3)	526(2)
In deep (fourth) layer	–	–	–	–	102(1)	–
Below deep layer	494(1)	–	–	–	1404(1)	–

[a] + = less than 0.5 individuals.
[b] – = no observations.

in the daytime from the main layer. The average catch per hour from immediately below this layer was about one-half that in it. Catches from immediately above the main layer, in the second layer, above the first layer, and in the deep layer, were all small. On the other two cruises the average catch per hour of fishing was again much greater in the main layer than above it; no hauls were made below the main layer (except for one deep haul to about 730 m). At night on Cruise '65-7 the average catches per hour from the main layer was about one-quarter the daytime catches. The catch from just below the main layer was the greatest but was somewhat less than the average daytime catch per hour from the same position relative to the layer, and less than the daytime catch from the same depth. The catch from the second layer was a little less than that from the main layer. On Cruise 64-6 the nighttime catch from the main layer was a little greater than the daytime catch but was only about one-half the night catch from the second layer. There was an appreciable catch from the surface layer. On Cruise 64-12 in late October the nighttime surface catch was the largest, much greater than that from either above or in the main layer. The average catch from just above the main layer was a little larger than that in it.

Species Obtained

The total fish catch from all three cruises, including the two deep tows, amounted to just over 10,600 individuals. In Table I are shown the depth and position of each haul with reference to the sound scattering layers and the composition of the catch by species, or in the case of less frequently occurring forms by family. Lanternfish (Myctophidae), by far the dominant group, made up about 81%; viperfish (Chauliodontidae) were 6%; deepsea smelt (Argentinidae) 4%; melamphids (Melamphaidae), almost entirely from the two deep tows, 2%; rockfishes (Scorpaenidae), about one-half pelagic juveniles, 2%; and flatfishes (Pleuronectidae), almost entirely larval forms, 2%. The remaining 2% was made up of individuals from 27 species in 21 families.

Of the eight species of lanternfish taken, four were common, *H. thompsoni*, *D. theta*, *Tarletonbeania crenularis*, and *L. leucopsarus*; and four were much less frequent, *Tarletonbeania taylori*, *Lampanyctus ritteri*, *Lampanyctus regalis*, and *Lampanyctus nannochir*. Depth distribution and vertical migration varied appreciably with species.

On Cruise 65-7 the largest catches of *H. thompsoni* were made in the daytime at 230–275 m in the main scattering layer. The numbers caught decreased progressively in the deeper hauls (275–320 m and 320–365 m) still within this layer. In the one haul from 230–275 m when the main layer lay below this depth range the numbers, although still relatively high were only one-third to one-half those when the layer lay in this depth range. Again, in 320–365 m, although the numbers involved are considerably smaller, more were taken when the layer lay at this depth than when it lay above it (90 individuals compared with 75). At night the largest catches of this species were made in about the same depth range as the largest daytime catches but were now from below the main layer. The numbers caught were only about one-third the daytime level. The catches in the main layer at 140–185 m were considerably larger than daytime catches at this depth, although smaller than those from 230–275 m and 275–320 m and below the main layer.

Catches of *Hierops* on Cruises 64-6 and 64-12 show the same general pattern of distribution. Although the numbers caught were considerably less than in 1965 and the depths fished somewhat shallower, down to about 275 m, more were again taken from the main layer than from above or below it. At night there was again an indication of movement towards the surface with the layer.

On Cruise 65-7 the largest catches of *D. theta* were two taken in the daytime at about 365 m, one from the main layer, the other from just below it. Of the two the former was the larger by about one-third. In the three hauls below 365 m daytime catches were small. Relatively large catches were also made in the two hauls in the main layer at 230–275 m. In the haul at this depth but above the main layer the catch was again small. None were taken shallower. At night the largest catch was from the main layer but at a depth of 140–185 m. The catch from the second layer at 90–140 m was also relatively

large, about one-half those in the main layer. In the two hauls from 275–320 m but below the main layer catches were about one-third as large as those in it.

On the other two cruises the largest daytime catches were again made in the main layer but from somewhat shallower depths (185–230 and 230–275 m). Only one tow was made to depths greater than 275 m; this was the deep tow to about 730 m. The number of *D. theta* caught was small. At night on both cruises more were taken at 90–140 m just above the main layer than in the main layer itself, and more were taken in the second layer than in the main layer. In October (Cruise 64-12) a large catch was made at the surface at night.

Lampanyctus leucopsarus was generally not as numerous in the catches as either *H. thompsoni* or *D. theta*, although in the deep tow to 510–595 m it provided the largest catch per hour of any species encountered. The largest individuals were most abundant in the two deep hauls to below 550 m. Excluding these hauls, more of this species were taken in the day in the main layer than above or below it, and more were taken when the layer was at about 230–275 m than when it was deeper. None were caught above 140 m in the daytime. At night an upwards movement apparently occurred. On Cruise 65-7 a concentration was found at night in the main layer at 140–185 m as compared with the daytime level of 230–275 m and some at 90–140 m. The numbers taken in the two hauls in 230–275 m but now below the main layer, although still relatively large, were less than in the daytime. On Cruise 64-6 there was a nighttime concentration in the second layer at 90–140 m and on Cruise 64-12 at the surface.

The numbers of *T. crenularis* taken were considerably smaller than of the three preceding species. However, once again more were generally taken from the main layer than from above or below it. In one case where significant numbers were taken below the main layer, over twice as many were taken in another haul from the same depth but in the main layer. There were indications of upwards dispersion at night.

Of the four less frequent species, it would appear from the small numbers taken that *T. taylori* resembled *T. crenularis* in distribution and *L. ritteri* resembled *L. leucopsarus*. *Lampanyctus regalis* may be a deeper water form than either *L. leucopsarus* or *L. ritteri*. It occurred mainly in the two deep daytime hauls and in the deeper nighttime hauls. *Lampanyctus nannochir* was only taken in two tows so little can be said about its distribution.

The viperfish *Chauliodus macouni* was the only species other than myctophids taken regularly in appreciable numbers. Although more were taken in the main layer than above it, the greatest numbers were taken during the day below this layer in depths of 365–455 m. At night fewer were taken below the main layer than in the day and some appeared in catches above the main layer and in the second and surface layers, showing there was considerable vertical migration.

Another stomiatoid, the melanostomiatid *Tactostoma macropus* was taken fairly frequently, mainly at night, or by day in deep hauls below 365 m. It

was apparently more abundant and had a shallower depth range in the late fall than in summer.

Five species of argentinid were taken. The two deep tows yielded not only all five species, but also the greatest quantities. In these hauls *Bathylagus milleri* and *B. pacificus* were numerous. The latter species also occurred in the deeper night tows to 230–275 m. *Bathylagus ochotensis* was taken on only one occasion during the day, but at night, especially on Cruise 64-12 (late October), it occurred from the surface downward. *Nansenia candida* was taken in the daytime mainly in hauls below 320 m whereas at night it appeared more numerous in the upper 140 m. Only a single specimen of *Leuroglossus stilbius* was taken, in one of the deep hauls.

DISCUSSION

The midwater trawl catches show that fish are associated with the sound scattering layers and that certain species are more numerous in these layers than elsewhere. The largest nighttime catches were usually made at somewhat shallower depths than the largest daytime catches. These diel changes in depth distribution coincide reasonably well with the changes observed in the daytime and nighttime positions of the sound scattering layers.

The experiments with broad spectrum sound sources have led to the hypothesis that the scatterers in sound scattering layers in the ocean are gas bubbles, probably the swim bladders of fish (Hersey et al., 1961). The systematic shift in peak frequency which accompanies a migration of a layer in depth further supports this hypothesis (Hersey and Backus, 1954; Johnson et al., 1956). The composition of the midwater trawl catches reported on here tends to lend additional support.

It is interesting to note that in the daytime 94% of the fish caught in the clearly defined persistent main scattering layer were lanternfish with well-developed swim bladders. On the other hand only 72% of the fish caught below the main scattering layer had swim bladders. Of these, 85% were of two species of lanternfish — *D. theta* and *L. leucopsarus* — known to have fat-infested swim bladders (Barham, 1957; Marshall, 1960). Based on the average numbers per hour's tow in and below the main layer, 89% of the fish with no swim bladders were taken in the latter position. Included were all the argentinids, all of the specimens of *Tactostoma macropus*, 83% of the viperfish, *Chauliodus macouni*, and 89% of the "other species" (mainly melamphids).

The intensity and persistence of the main scattering layer may thus be caused by the presence in it of a higher proportion of fish with swim bladders than without, whereas the presence of faint, intermittent layers below it where relatively large catches suggested that fish were still abundant may be caused by the prevalence of fish with no swim bladders or with modified swim bladders. Unfortunately it is not known whether fat-infested swim bladders are as effective sound scatterers as are gas-filled bladders.

In the daytime few fish were taken above the main scattering layer, i.e. above 230–275 m. On Cruise 65-7 tows were made during the day in both

the first and second scattering layers, lying at about 90 m and 185 m, respectively. These layers were generally less intense and more intermittent than the main layer. Catches were small or nil. It would appear either that fish were not associated with these layers in the daytime, or if they were, avoided capture in the higher light level of the shallower water. Pearcy and Laurs (1966) found that catches of mesopelagic fish off Oregon were larger at night than in the day in the 0–150 m depth interval, larger in the day than at night in the 150–500 m interval and about equal in the 500–1000 m interval. The night/day ratios were 6:1, 0:6, and 1:1, respectively. They showed the increased surface catches at night were due in part to vertical migration and in part to net avoidance. The effect of each varied with species. They considered vertical migration explained the major portion of the surface increase of *D. theta* at night, but that most of the nighttime surface increase of *L. leucopsarus*, *T. crenularis*, and *T. macropus* were due to net avoidance. However, in the present case it does not seem reasonable to account for the almost complete lack of daytime catches above 185 m (Table I) in a net with the large frontal area of the Engel trawl[4] on the basis of net avoidance. It would seem more likely that fish were not associated to any extent with these upper layers in the daytime. This explanation is supported by Tucker's (1951) conclusion that during the day the upper (500–800 ft deep, i.e. about 150–240 m) and less intense of two scattering layers was related to a euphausiid population, whereas the lower (800–1000 ft deep, i.e. about 240–305 m) and more intense layer was related to a fish (myctophid) population. In contrast, at night the catches indicate that fish were associated with these upper layers, although not in the numbers found in the main layer. That part of the main layer which split off and ascended to merge with or pass through the second layer thus may have consisted of fish. Our catches provide no information on the possible invertebrate composition of any of the layers observed, or on the vertical movement of such organisms.

Aron (1962) reported that midwater tows within the upper 400 m in the northeastern Pacific yielded a larger number of fish at night than during the day. Likewise Hersey and Backus (1962) found that in the upper 300 m night catches of myctophids south of New England were higher than day catches. On the other hand, off San Diego, California, Tucker (1951) demonstrated the reverse, that day catches were larger than night catches. Results from the third cruise, 65-7, bear out Tucker's observation, whereas those from the other two cruises would appear to indicate that more fish were taken at night than during the day. However, if the depth of the net is not regulated so it fishes well within the boundaries of a layer, some fish may be missed and the catches would be low. It is probable that some of the low daytime catches on the first cruise were the result of not sufficiently precise

[4]The shape the mouth of the Engel trawl assumes when towed is not known. The frontal area given by the product of the lengths of the headline and breastline is 1330 m^2, by the product of the headline and the vertical height attained when towed is 570 m^2. Because of the possible effect of mesh selectivity, the effective frontal area could be less than either. The frontal area of an Isaacs-Kidd trawl used by Pearcy and Laurs (1966) is given as 2.89 m^2.

regulation of the depth of the net. When individual rather than average catches are considered on this cruise, the largest day catches were bigger than the largest night catches. It is possible that on this cruise too, day catches should be larger than night catches. On the second cruise, 64-12, in late October it appeared night catches were larger than day catches; however, only five hauls were made, two by day and three at night, and hauls were not made to below 230 m. Although it is possible the maximum daytime concentration was missed, this is not likely, for the main layer appeared to be shallower than in summer and well within the depth zone fished.

The concentration of fish in the main scattering layer during the daytime and their more dispersed distribution at night, with numbers being taken from the shallower layers where they were not present during the day indicate that the fish associated with the layers migrate vertically in the early evening. Examination of the diel distribution of the more common species shows differences in the migration patterns. Of the four more common lanternfish, *L. leucopsarus* appears to make the most extensive migrations and *H. thompsoni* the least. Interestingly, *L. leucopsarus* and *D. theta*, forms known to have fat-infested swim bladders make relatively extensive vertical migrations. On the other hand, *H. thompsoni*, a form with a gas-filled bladder, appears to make a less extensive migration although it is dispersed upwards throughout the water column after dusk. It was pointed out that whereas both *H. thompsoni* and *D. theta* are most numerous within the main layer, *D. theta* tends to occur at greater depths (320–365 m) than *H. thompsoni* (230–275 m). *Lampanyctus leucopsarus* has a greater depth range than either of the above two species. During the day the main centre of distribution seems to be below 455–595 m with a secondary centre at 230–275 m, primarily of smaller individuals. At night it becomes more generally dispersed throughout the water column with more taken in the upper 185 m than between 185 and 365 m. Barham (1957) indicates that in Monterey Bay, *L. leucopsarus* is associated with a somewhat deeper scattering than *D. theta*.

Some of the fish without swim-bladders, particularly the argentinids and *T. macropus*, appear to undertake extensive vertical migrations. During the day these forms were absent from the upper 320 m and appeared to be concentrated below 550 m. At night no concentrations were located, but individuals were taken not only between 230 and 320 m but also in the upper 90–140 m. Similarly, during the day few viperfish (*Chauliodus macouni*) were taken above 280 m and they appeared to be most numerous at about 455 m. At night they were taken quite regularly in the upper 185 m.

The composition of the midwater trawl hauls suggest: (1) that fish, particularly those with swim bladders, are found in numbers in the main scattering layer and undertake vertical migrations in conjunction with this layer; (2) that fish without swim bladders or with modified swim bladders (fat-infested) although occurring in the main layer may be more numerous below this layer and may undertake more extensive vertical movements than fish with swim bladders; and (3) that fish with fat-infested swim bladders may be less effective sound scattering than those with gas-filled bladders.

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Blood Lactate Concentrations and Mortality in Sockeye and Chinook Salmon (*Oncorhynchus nerka* and *O. tshawytscha*) After Exercise[1]

By William D. Gronlund, Harold O. Hodgins, Raymond C. Simon,[2] and Douglas D. Weber

U.S. Bureau of Commercial Fisheries
Biological Laboratory, Seattle, Wash.

ABSTRACT

Blood lactate concentrations in 306 sockeye salmon (*Oncorhynchus nerka*), taken at sea in 1965 and 1966 by purse seine, gillnet, and longline, and in 98 starved or fed immature chinook salmon (*O. tshawytscha*) that had been exercised in a saltwater pond were generally below those reported in the literature as related to stress-induced mortality in salmon. No sockeye salmon held in shipboard tanks for observation died within 2 days after capture, although most of the fish died later. Few fish died among starved or fed immature chinook salmon in salt water within 10 days after capture by hook and line or after being chased to exhaustion; blood lactate concentrations in these fish were generally higher than in sockeye salmon after stress but were still usually below the reported critical level of 125 mg/100 ml.

We concluded that neither the adult sockeye nor immature chinook salmon suffered immediate fatigue-related mortality, but the possibility that fatigue could induce delayed mortality was not excluded.

INTRODUCTION

Many reports have been published during the past 30 years on the relation between severe muscular exercise and mortality in fishes; comprehensive reviews appear in Black (1958), Beamish (1966), and Black et al. (1966). Among the salmonids, mortality attributed to muscular fatigue and related stress were reported for chinook salmon (*Oncorhynchus tshawytscha*) by Parker and Black (1959); for coho salmon (*O. kisutch*) by Milne and Ball (1956) and Parker et al. (1959); for sockeye salmon (*O. nerka*) by Black (1957c); and for rainbow trout (*Salmo gairdneri*) by Bouck and Ball (1966). In contrast, no mortalities after severe exercise were reported for chinook salmon by Jensen (MS, 1958); for coho salmon by Paulik et al. (MS, 1957) and Parker et al. (1959); for sockeye salmon by Paulik and DeLacy (MS, 1958); for Kamloops trout (*S. gairdneri*) by Black (1957a); nor for lake trout (*Salvelinus namaycush*) by Black (1957b).

Why mortality occurred in some groups of fish after extreme exercise and not in others has not been clearly shown. Beamish (1966) noted that fatigued coho, chinook, and sockeye salmon that died were in salt water, whereas groups in which mortality was insignificant after severe exercise had

[1]Received for publication September 6, 1967.
[2]Present address (R.C.S.): Bureau of Sport Fisheries and Wildlife, Oregon State University, Corvallis, Ore.

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been starved and were exercised in fresh water. Jensen (MS, 1958), however, observed little mortality in age-II and age-III chinook salmon caught by hook and line in salt water. In immature troll-caught chinook salmon, Parker and Black (1959) demonstrated a positive correlation between mortality and concentrations of blood lactate.

Parker et al. (1959) also reported high blood lactate concentrations and a high mortality for feeding coho salmon captured by ocean trolling. They found also that adult coho salmon displayed neither high mortality nor high concentrations of blood lactate after exertion in fresh water. They concluded that troll-caught coho salmon suffered mortality when blood lactate concentrations exceeded 125 mg/100 ml and remained elevated during holding after capture. However, the specific cause of death may have been acidosis from *p*H changes (Jonas et al., 1962) rather than lactic acid concentration per se.

Factors other than severe injury or fatigue may also be related to mortality or disability of salmonids after capture and handling. Parker et al. (1963), for example, speculated that delayed mortalities may arise from breaks in the epidermis or from scale loss, which result in secondary infections or osmotic imbalance.

Most reports on fatigue-related mortality are concerned with death within several hours of stress (here termed "immediate" mortality). Little work has been done on delayed effects. Milne and Ball (1956) and Bouck and Ball (1966), however, observed delayed mortality (death after more than 1 day) after stress in coho salmon and rainbow trout.

The present studies were initiated to provide information on mortality or disability as a result of stress imposed on salmon during capture and tagging at sea and to define better the mechanisms involved. Sockeye salmon caught on the high seas were sampled to assess the effects of fatigue, as induced under field conditions, on blood lactate levels and mortality. Chinook salmon reared in a hatchery were held in a saltwater pond to determine the incidence of delayed mortality and to compare mortalities of fed and starved fish after known periods of exercise.

METHODS AND MATERIALS

Collection Procedures and Sampling Methods for Sockeye Salmon

Maturing salmon from Bristol Bay, Alaska, were sampled during June and early July of 1965 and 1966 in "offshore" and "inshore" areas (Fig. 1). Our sampling in 1965 was set up to assess the lactate response of sockeye salmon during field tagging procedures. The design was modified in 1966 to include sampling of blood over a longer period after landing and to observe mortality after capture.

Purse seines were used in 1965; each set lasted 30–45 min. Salmon were brailed from the seine and placed in a square plywood tank (4100 liters) supplied with a continuous flow of fresh sea water. Not more than 550 fish were held at any time. Unloading the seine usually took about 10 min. The landing

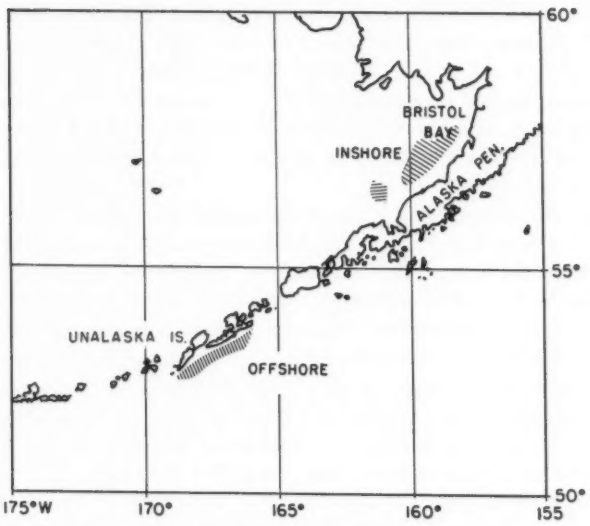

FIG. 1. General sampling locations for Bristol Bay sockeye salmon in 1965 and 1966.

time for a group of fish was taken as the time the first net load was brought on board. Fish to be sampled for physiological studies were dipped out of the holding tank and quickly killed by a sharp blow on the head. A total of 139 fish were sampled from 5 to 140 min after landing; the rest were tagged and released. Blood was obtained by cardiac puncture and collected in sterile tubes without anticoagulant. A 0.5-ml aliquot of whole blood was immediately added to 4.5 ml of cold 10% trichloroacetic acid (TCA) solution and placed at −18 C in a shipboard freezer for subsequent laboratory analysis for lactate. The length, sex, and general condition were then determined for each fish. Surface water temperatures varied from 3.8 to 6.8 C during the sampling period.

Gillnets and longlines were used for capture in 1966, but most blood samples were from fish taken on longlines. The longline gear was fished for 1 hr in all offshore sets and for 1½ hr at the single set made inshore. All but one of the sockeye salmon captured by longline for these studies were alive when landed and appeared healthy and uninjured. As the gear was retrieved, hooks were removed and the fish were placed in a canvas holding tank 1 m in diameter; salt water flowed through the tank continuously and the water was 1 m deep. No more than five fish were in the tank at once.

One of every three sockeye salmon brought on board was killed and blood was collected as in 1965, except that an anticoagulant (ammonium heparin) was used; a total of 71 fish were sampled. An aliquot of each blood sample was placed in TCA for lactate determinations; the remainder was

centrifuged and plasma collected for other studies. The other fish caught were tagged with Petersen discs and placed in one or the other of two steel holding tanks (each 0.9 × 2.1 × 0.75 m deep) through which a constant flow of fresh sea water was pumped to ensure adequate aeration. Ocean surface water temperatures varied from 3.8 to 6.3 C. The fish (96) from one tank were killed from 2 to 6 hr after landing and sampled as above. The remaining 39 fish were held for mortality estimates.

Samples for blood lactate determinations were taken from three other groups of fish. Four sockeye and 10 chum salmon (*O. keta*) caught by longline were held and the same individuals bled repeatedly for blood lactate determinations; in this serial sampling, blood was taken from the ventral aorta where possible, or otherwise from the heart. These fish were placed in a tagging cradle, ventral side up, and manually held. No difficulty was experienced and most samples were obtained within 20 sec. The second group was composed of 25 sockeye salmon which had been held for 3–52 hr for mortality studies. These fish were severely stressed by accidental draining of the holding tank because of water pump failure; they struggled intensely, lost many scales, and most were motionless by the time the water was restored 20 min later. All fish subsequently revived, but a few failed to maintain an upright position. All were killed and sampled for blood lactate 1½–2 hr or 3½–4½ hr after the water supply failed. The third group of fish for which blood lactates were determined consisted of 17 gillnetted sockeye salmon; these fish were initially selected because they appeared to be vigorous and uninjured and were placed in the steel holding tanks to recover. After 12–14 hr or 36–38 hr, the fish were killed and blood samples taken.

Blood lactate was determined by the colorimetric method of Barker and Summerson (1941), as outlined in Oser (1965, p. 1102–1105). Repeated determinations (27) made on a standard solution (50 mg/100 ml lithium lactate) established that this technique was accurate (range, 44.9–54.2 mg/100 ml). The maximum range of values from multiple bleedings of 10 sockeye salmon was ±5.4 mg/100 ml around the mean.

Sampling Procedures for Chinook Salmon

Fall chinook salmon of the 1964 year-class from hatcheries on the lower Columbia River were transferred to salt water at the Bowman's Bay marine station of the Washington State Department of Fisheries in June 1965. The fish, which ranged from 23 to 37 cm fork length, were fed Oregon moist pellets three times a week. Water temperatures ranged from 6 to 9 C. Mortality, mainly of precocious males, was about 3% during November 1966; our experiments were conducted in December 1966.

The fish were herded without handling into partitioned areas of a pond (Fig. 2), where they were separated into fed and starved test groups (to be exercised) and a control group (to be fed, but not exercised). The two test groups contained about 60 fish each, the control group over 200. Fish in our first experiment were fatigued by capture on hook and line. The procedure

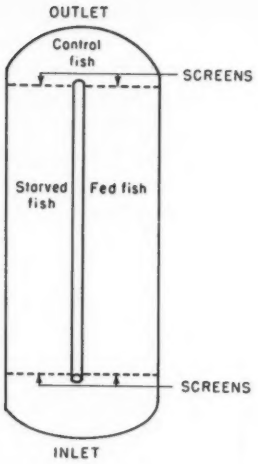

FIG. 2. Diagrammatic view of saltwater rearing pond (6 × 24 m) in which chinook salmon were tested.

was similar to that used by Jensen (MS, 1958) except that our fish were tagged instead of finclipped. Fourteen fish (8 fed, 6 starved) were caught, tagged with Petersen discs, and released. In later experiments, water in the pond was lowered to 7–8 cm; the fed and unfed test groups in the two raceways were then simultaneously chased to exhaustion (18 min) and could be picked up easily.

Twelve fish from each exercised test group were tagged and returned to the water, which was restored to a depth of about 1 m, to assess the combined effects of exercise and tagging; the remaining 40–42 fish from each test group were left untagged to determine the effects of exercise alone. The water level for the control group (about 200 fish) was never lowered below 0.3 m in the well at the end of the pond, where they were disturbed as little as possible. Mortalities for the three groups were monitored for 10 days after exercise by daily inspection and carcass collection.

In a later experiment, 60 fish were taken randomly from the 280 control and remaining test fish (those that were chased but not handled or tagged) of previous experiments. These 60 fish were separated and chased to exhaustion as before. About half were killed for blood lactate determinations at intervals of $1\frac{1}{2}$–2 hr and 5–$5\frac{1}{2}$ hr after they had been chased. The rest were held and mortality observed. An unexercised group of 22 fish was also sampled to determine blood lactate concentrations while at rest.

EFFECTS OF STRESS

BLOOD LACTATE IN SOCKEYE SALMON

For two groups of sockeye salmon caught by gillnets and held in shipboard tanks, blood lactate values 12–14 hr after capture (12.6 ± 1.10 mg/100 ml; n = 10) were similar to those after 36–38 hr (9.6 ± 1.34 mg/100 ml;

n = 7). These results are consistent with resting values previously reported for salmon (Black, 1957c).

Initial blood lactate concentrations of 139 sockeye salmon sampled from 5 to 130 min after capture by purse seine (Fig. 3) were only slightly higher (13–18 mg/100 ml) than resting values (9–12 mg/100 ml). The concentrations for fish caught offshore rose to 42 mg/100 ml after 110 min, whereas those of inshore fish rose to 30 mg/100 ml of blood. For both groups the concentrations after 110 min were more than double the initial values.

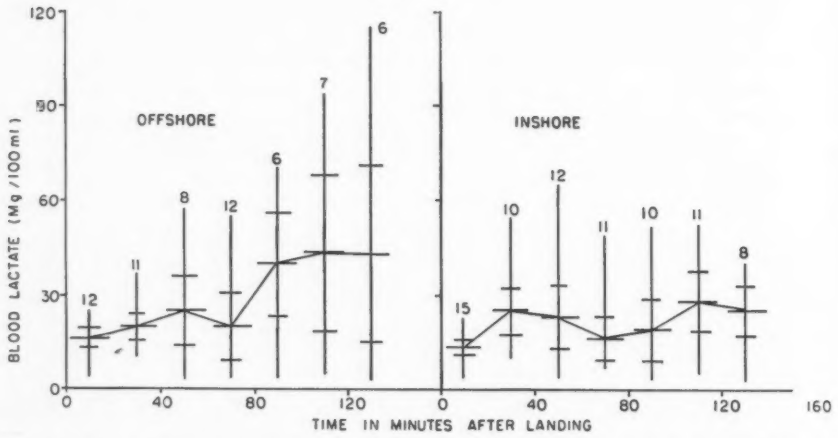

FIG. 3. Blood lactate concentrations in sockeye salmon captured by purse seine in 1965. Vertical bar shows total range; short horizontal bars above and below the mean show upper and lower 95% confidence limits; number above vertical bar shows number of fish in sample.

Blood lactate concentrations of 167 fish, captured by longline and sampled up to 380 min later (Fig. 4), were 2–5 times as high as the values for fish captured by purse seine at comparable times. In offshore fish, the initial concentration of about 50 mg/100 ml increased to 75 mg/100 ml after 140 min and subsequently declined toward resting values after 300 min. The inshore fish had higher initial blood lactate concentrations (62 mg/100 ml) than the other groups studied and the subsequent values were generally higher; however, the values were approaching resting levels after 380 min. These higher concentrations in fish sampled inshore probably reflected more severe weather conditions during sampling than were experienced at other times.

Mean concentrations of blood lactate in 25 sockeye salmon that were severely stressed by draining the tank (Table I) were higher (136–141 mg/100 ml) for fish held only a short time after capture before secondary stress than for fish allowed longer periods of recovery (71–94 mg/100 ml). These results agree with observations on the additive effects of repeated stress on blood lactate concentrations in rainbow trout reported by Stevens and Black (1966).

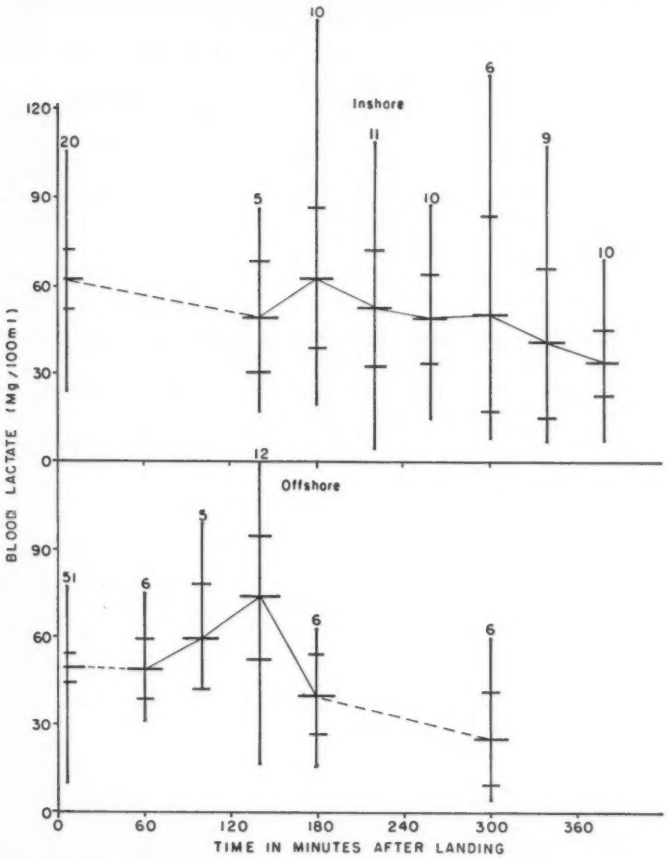

FIG. 4. Blood lactate concentrations in sockeye salmon captured by longline in 1966; broken line indicates incomplete data. Vertical bar shows total range; short horizontal bars above and below the mean show upper and lower 95% confidence limits; number above vertical bar shows number of fish in sample.

Lactate concentrations from serially bled fish captured by longline (Table II) were 60–120% higher for chum than for sockeye salmon in blood taken more than 1 hr after capture. Because the conditions of stress were similar, this may indicate a difference between species. The added stress of serial bleeding is not reflected in blood lactate concentration as these values for sockeye salmon were consistently near or below those for singly bled fish at comparable times (Fig. 4).

TABLE I. Effects of stress from draining shipboard holding tank on levels of blood lactate in maturing sockeye salmon.

Hours spent by fish in tank before draining	Hours after draining and refilling tank before sampling	No. of fish	Blood lactate (mg/100 ml) mean ± SE
3–4½	1½–2	7	141±10.4
3–4½	3½–4½	7	136±21.1
27–52½	1½–2	5	94±4.0
27–52½	3½–4½	6	71±7.6

TABLE II. Levels of blood lactate from serial bleedings of maturing sockeye and chum salmon held in shipboard tanks.

Species	No. of fish	Time of blood sample collection (*hr after capture*)	Blood lactate (mg/100 ml) mean ± SE
Sockeye			
	4	0–¼	41.2±6.08
	4	2–3¼	51.5±13.48
	4	5¾–11¼	13.3±4.06
Chum			
	10	0–¼	41.1±4.78
	10	1–3½	81.6±13.62
	9[a]	5¼–9¼	28.8±7.91

[a]One fish died after 3½ hr.

MORTALITY IN SOCKEYE SALMON

No mortality occurred within 48 hr in sockeye salmon held in shipboard tanks (Table III). Mortality began after 2 days but was probably caused by shipboard holding. The development of numerous surface lesions on these fish after 2 days indicated that disease was associated with the mortality. Fish captured inshore died more rapidly than those captured offshore, but this difference may have been a result of greater stress caused by rougher seas at the inshore station.

BLOOD LACTATE AND MORTALITY IN CHINOOK SALMON

Within 10 days after exercise two tagged test fish caught by hook and line and two control fish had died (Table IV). One of the tagged fish that died received a severe hooking injury and did not recover; the other died 2 days after exercise. Both of the dead control fish were precocious males.

Blood lactate concentrations of exercised and unexercised chinook salmon (Fig. 5) were typical of those reported in the literature cited previously: mean

TABLE III. Mortalities of maturing sockeye salmon captured by longline and held in shipboard tanks.

Days after capture	Inshore		Offshore	
	No. dead	Cumulative mortality (%)	No. dead	Cumulative mortality (%)
0	0	0	0	0
1	0	0	0	0
2	0	0	0	0
3	4	16	0	0
4	8	48	1	7
5	2	56	0	
6	0	56	0	
7	10	96	0	
8	0	96	0	
9	1	100	1	14
10			1	21
11			0	
12			3	43
13			0	
14			0	
15			0	
16			2	57
17			2	71
18			0	
19			0	
20			1	79
21			0	
22			0	
23			0	
24			2	93
25			0	[a]
Totals	25	100	13	93

[a]One fish alive on 25th day.

concentration rose to 110 mg/100 ml about 2 hr after exercise and then declined about 5 hr after exercise. Concentrations were extremely high in a few individuals, but none died within 3 days after exercise.

RELATIONS BETWEEN STRESS, BLOOD LACTATE, AND MORTALITY

These studies indicated that in maturing Bristol Bay sockeye salmon in the marine environment the blood lactate response to stress was similar in timing to that reported for other Pacific salmon. But the concentrations attained under the conditions imposed were below those in troll-caught oceanic chinook and coho salmon which experienced stress-related mortality (Parker

TABLE IV. Mortalities of 2–2½-year-old chinook salmon after different types of stress.

No. of fish	Stress: Nature	Stress: Days after feeding	Stress: Duration (*min*)	No. of deaths within 10 days
8	Hook & line + tagging	1	3.7–6.5	0
6	Hook & line + tagging	8	2.5–5.0	2[a]
12	Chasing + tagging	1	18	0
12	Chasing +tagging	15	18	0
40	Chasing	1	18	0
40	Chasing	15	18	0
200	None	1		2[b]

[a]One fish suffered severe hooking injury and did not recover; the other died 2 days after hooking and tagging.
[b]Both fish that died were precocious males.

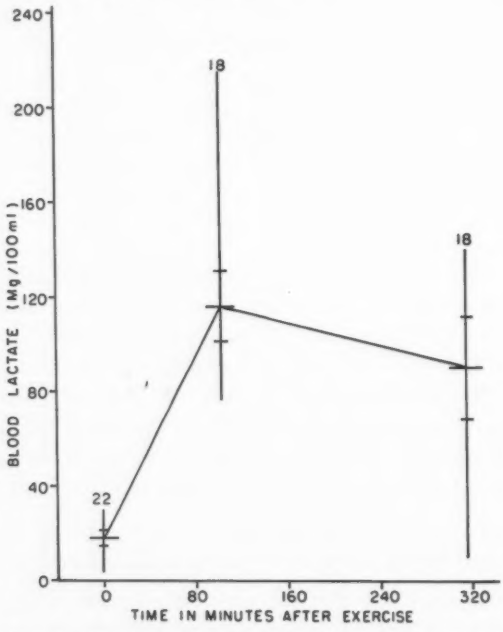

FIG. 5. Blood lactate concentrations in non-exercised chinook salmon and in those chased to exhaustion. Vertical bar shows total range; short horizontal bars above and below the mean show upper and lower 95% confidence limits; number above vertical bar shows number of fish in sample.

and Black, 1959; Parker et al., 1959). Immediate mortality did not occur in the present studies. Possibly the levels or types of stress imposed were not comparable.

Bristol Bay sockeye salmon, if subject to sufficient stress, were shown (Table I) to be capable of developing the concentration of blood lactate designated as critical (125 mg/100 ml) by Parker et al. (1959). Even after the severe trauma experienced by fish when the holding tank was drained, however, no fish died before we sampled for lactate $1\frac{1}{2}$–$4\frac{1}{2}$ hr later.

Examination of stomachs confirmed that the inshore and offshore groups of sockeye salmon were actively feeding. The observed lack of deaths cannot be related, therefore, to cessation of feeding.

The series of experiments performed on chinook salmon in the saltwater pond failed to demonstrate that mortality, either immediate or delayed, was associated with exercise or handling in either starved or fed fish. Blood lactate concentrations were higher than those observed in Bristol Bay sockeye salmon, but were still generally below the critical levels reported elsewhere.

The stress of capture and tagging, therefore, caused no immediate mortality in any of the salmon groups we studied. The delayed deaths of sockeye salmon in shipboard tanks conceivably could have been caused by the stress of initial capture instead of confinement, but we found no delayed mortality in pond-reared immature chinook salmon. The results of pond experiments on immature fish of one species, however, are not necessarily valid for maturing salmon of another species on the high seas.

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Behavior and Distribution of Spawning Sockeye Salmon on Island Beaches in Iliamna Lake, Alaska, 1965[1,2]

By Orra E. Kerns, Jr., and John R. Donaldson[3]

Fisheries Research Institute, College of Fisheries
University of Washington, Seattle, Wash.

ABSTRACT

In 1965, spawning was studied on the island beaches of the eastern part of Iliamna Lake from a 5.5-m tower mounted on a 9.1-m boat and with the use of scuba. Over 3 million sockeye salmon, *Oncorhynchus nerka* (Walbaum), utilized nearly 130 ha of the beaches surveyed during the period August 8–21. The total spawning population and utilized area of shoreline of all the islands in the lake were greater since each area was surveyed only once and possible multiple waves of spawners were not taken into account, and not all of the island shoreline was surveyed by the above methods. The salmon generally spawned in dense groups, over immovable rock, in exposed areas with no upwelling ground water, and with little display of territorial defense. Density ranged from less than 0.1 fish to more than 5 fish per 0.84 m^2 (1 $yard^2$). The latter density prevailed on reefs and outside points of the islands. Seventy-three per cent of spawning took place over bottom with irregular rocks from 102 to 305 mm (4–12 inches) in diam, and over 90% at depths from 2 to 6 m (6–20 ft) on bottom with slopes from 15 to 25°. Commonly, in a group of fish, one or more of the females were seen with their vents down in rock interstices, emitting eggs, while the males in close proximity were observed releasing milt. Eggs on the beaches are probably aerated by wind-generated lake currents and seiches. Of 3553 eggs collected from several island beaches, 6.8% were infertile.

Several features of the spawning behavior and environment are favorable to the survival of eggs and fry. The bottom is not scoured. Most eggs are deposited between immovable rocks and are thus not disturbed by subsequent spawners. Fry emerging from the beaches have immediate access to the large food supply in the lake. Some hazards are predation on eggs by fishes, freezing, and ultraviolet radiation of eggs in shallow water, and smothering of eggs in areas with a high density of eggs or algal growth.

INTRODUCTION

The typical spawning behavior of sockeye salmon, *Oncorhynchus nerka* (Walbaum), throughout its range can be described as follows. The female selects and digs a redd in gravel in which the eggs are deposited. An accompanying male fertilizes the eggs. The female defends the territory around the redd during and after spawning. The selected gravel is movable by the female and contains subsurface flowing water. This behavior is characteristic of sockeye salmon spawning in streams, in spring ponds, and on mainland beaches of Iliamna Lake, but not of those spawning on the island beaches. This paper describes the unique behavior and distribution of spawners on island beaches in 1965.

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[1]Received for publication June 29, 1967.
[2]Contribution No. 278, College of Fisheries, University of Washington, Seattle, Wash.
[3]Present address (J.R.D.): Department of Fisheries and Wildlife, Oregon State University, Corvallis, Oregon.

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Iliamna Lake is the second largest freshwater lake in the United States exclusive of the Great Lakes (Bue, 1963). It has a surface area of 2622 km² (Lenarz, 1965) and over 250 km of island shoreline. Most of the islands are in the eastern and deepest end of the lake (Fig. 1), and only a few are in the western end, which is comparatively shallow and uniform in depth. The lake is connected to Bristol Bay by the 92-km long Kvichak River, the lower 69 km of which are tidal. The Kvichak River watershed encompasses nearly 21,000 km² (U.S. Army Corps of Engineers, 1957).

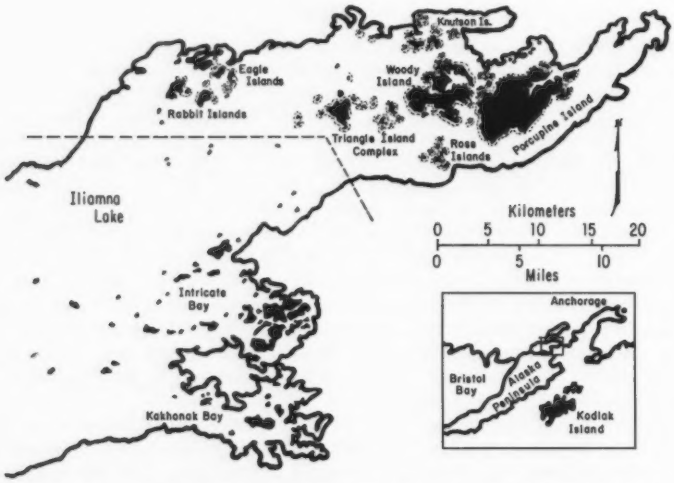

FIG. 1. Spawning areas on island beaches of eastern part of Iliamna Lake (indicated by broken lines) as observed from boat tower in August 1965. Observations were not conducted south and west of broken line across lake.

The annual run of sockeye salmon to the Kvichak River system varies in magnitude with cyclic regularity and is the largest contributor to the Bristol Bay fishery. Since 1955, when an accurate count of the escapement was initiated by the Fisheries Research Institute, the total run has varied from 0.56 million (in 1963) to 42.1 million (in 1965) (Ossiander, 1966). In 1965, a peak cycle year, 24.3 million sockeye escaped to the spawning grounds (Pennoyer and Seibel, 1966).

Early studies demonstrated the importance of the island spawning grounds in some years and supplied information on some features of the biology of the salmon and physical aspects of the spawning grounds. Aerial surveys by Fisheries Research Institute personnel of the spawning grounds each season since 1955 have shown the much greater percentage of spawning on island beaches in peak years of the cycle than in off years. Spawning was observed closely by using scuba on some islands in 1960 and 1961, earlier peak and

subpeak cycle years, respectively (Kerns, 1961, 1962). Olsen (MS, 1964) reported physical differences between an island and a mainland beach of Iliamna Lake as well as differences in the timing of spawning and the development of eggs from the 1962 and 1963 spawning.

Because of the large peak year escapement, spawning on island beaches was extensively studied in 1965. The objectives were to determine the distribution and density of spawners, to describe spawning behavior, and to assess spawning success.

MATERIALS AND METHODS

The distribution and density of spawners were observed from a portable tower mounted on the cabin of a 9.1-m boat (Fig. 2). The tower platform was 5.5 m above the water surface. A steering arrangement allowed two observers on the platform to maneuver the boat to depths as shallow as 1 m.

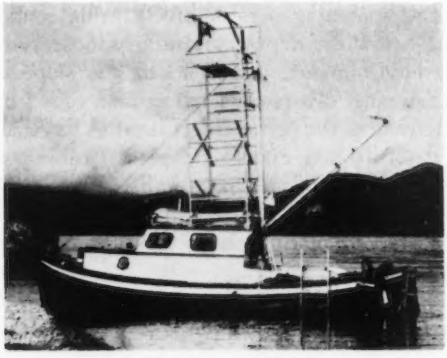

FIG. 2. Observation tower mounted on vessel, Iliamna Lake, 1965.

The boat speed during observations was approximately 8 km per hour. When the water surface was calm it was possible to see salmon at depths up to 11 m and at distances up to 15 m on either side of the boat. Since the littoral shelf seldom exceeds a depth of 6 m and a width of 30 m, it was possible to cruise at distances from shore ranging from 15 to 30 m and observe all spawners. Several parallel passes were made along wide beaches and broad reefs. Observations were made from August 8 to August 21, and each beach was observed only once.

During each survey one observer steered the boat while the other recorded the following information on a large-scale map of the survey area from visual observation: density of spawners (number of fish per yard^2); width and depth of area occupied by spawners; type of bottom; slope of beach; state of spawning.

The frame of reference for the last observation was as follows: *spawning* is the period extending from arrival of the fish and dispersal on a spawning beach until their death. There are three stages of spawning: *prepeak*, when the majority of the fish have not deposited eggs or milt; *peak*, when the majority are depositing eggs or milt; and *postpeak*, when the majority have deposited eggs or milt. These stages of spawning were estimated by fish behavior and color, which will be discussed later. The relationship between stage of spawning and fish behavior and color had been established by considerable sampling over a period of years.

Coded colors and stippling were used on maps to represent densities of spawners ranging from less than 0.1 fish to more than 5 fish per 0.84 m^2 (1 $yard^2$). Proficiency in estimating densities was acquired by observers by first counting fish over known areas of bottom at different densities. Duplicate counts were made by each observer over various densities to measure bias. It was found insignificant. The extent of a spawning area along a beach was calculated from maps. The number of spawning fish on a particular portion of a beach was computed from area utilized and density.

During the peak spawning period, divers using scuba checked the possibility of spawning below the depth of visibility, observed and photographed the fish and their environment, and checked the slope of bottom. Several weeks after peak spawning, divers collected salmon eggs from several locations on the beaches to determine the per cent fertilized. A large rubber bulb attached to a 200-mm length of 10-mm diam rigid plastic tubing was used to collect the eggs from the interstices of the rocks and place them in a plastic bottle. Eggs were collected from single large deposits as well as randomly over a large area. They were preserved in Stockard's solution (5% formalin, 4% acetic acid, 6% glycerine, and 85% water).

Some aerial and boat surveys were made of prespawning fish that were transiently schooled, usually along shoreline not utilized for spawning.

DISTRIBUTION AND DENSITY OF SPAWNERS

Some 84 km (about 130 ha) of the 126 km of island shoreline surveyed from the boat tower (Fig. 1) were utilized for spawning. The estimated population was 3 million spawners (Table I). The spawning population of all the islands in the lake was considerably greater than this estimate. Several spawning areas in the open portion of the eastern part of the lake were occupied by spawners during aerial surveys but were not surveyed from the boat tower because of inclement weather. Further, the islands of Intricate and Kakhonak bays were not observed from the boat tower; aerial surveys of these islands showed utilization of some beaches. Also, each area was surveyed by boat only once, and possible multiple waves of spawners during the spawning season were not taken into account.

When the beaches were surveyed, approximately 26%, 53%, and 21% contained fish in the prepeak, peak, and postpeak stages of spawning respectively.

TABLE I. Areas utilized and abundance of sockeye spawners on island beaches surveyed from the boat tower, Iliamna Lake, 1965.

Spawning area	Distance utilized (*km*)	Area utilized (*ha*)	Estimated no. of fish
Woody Island	29.0	49.8	1,015,000
Porcupine Island	21.9	23.8	617,000
Ross Islands	5.3	7.2	173,000
Triangle Island complex	7.6	20.2	638,000
Rabbit and Eagle islands	7.2	8.8	147,000
Knutson Bay Islands	13.2	19.4	432,000
Total	84.2	129.2	3,022,000

Distribution maps were prepared from the maps made during each survey, such as the one shown for Woody Island (Fig. 3). Spawners were found on nearly every portion of the outside beaches of the island. Few spawners were found on the southwest beach, but more than five fish per 0.84 m^2 (1 $yard^2$) were observed on narrows and reefs of the northern and eastern portions of the island.

Generally, the beach spawning took place largely in the eastern end of the lake and densities of spawners were heaviest on exposed points of land at narrows between islands and on reefs. It occurred in areas where no upwelling ground water has been recorded to date. Olsen (MS, 1964) found equal lake and intergravel temperatures during the spawning season and concluded that

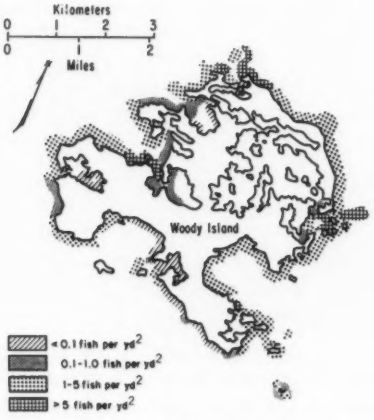

FIG. 3. Densities of sockeye salmon spawners on Woody Island, Iliamna Lake, Alaska, August 1965. (Width of stippling does not indicate width of spawning area utilized.)

little, if any, upwelling ground water occurs. Studies in Great Central Lake on Vancouver Island, B.C., revealed three sources of water movement in spawning gravel: upwelling ground water, changes in lake level, and waves (Fisheries Research Board of Canada, 1961, p. 98). Observations of some sockeye spawning beaches in Lake Washington of Washington State led Woodey (MS, 1966) to the conclusion that little or no ground water occurs on some spawning beaches and that water circulation in redds is accomplished by wind-driven waves and lake currents. Olsen (MS, 1964) concluded that on an island spawning beach of Iliamna Lake, wind-generated lake currents and seiches are the means of water circulation.

Donaldson (1966) reported that, for a basin the size of Iliamna Lake, the theoretical periodicity of a unimodal seiche along the long axis is 3.3 hr, and that empirical measurements of the seiche in Iliamna Lake also gave a periodicity of 3.3 hr. The average amplitude of the seiches measured at the Porcupine Island station was 11.4 mm in 1965. During periods of strong winds, however, the seiche amplitude sometimes reached 51 mm. The total vertical movement of water, therefore, averages over 25 mm with a maximum of over 100 mm.

Ebb-and-flow currents due to the action of wind and seiche are present in narrows between islands of the eastern part of the lake. Their velocity, either at the lake surface or within the substrate, has not been measured.

During the survey, four types of spawning bottom were distinguished: (1) *Coarse gravel*: a mixture of rocks 51–102 mm (2 to 4 inches) in diam with little or no sand. The salmon can move these rocks, but they do not appear to do so. (2) *Small broken rock*: irregular rocks from 102 to 305 mm (4 to 12 inches) in diam, which the salmon cannot move. (3) *Large broken rock*: irregular rocks greater than 305 mm (12 inches) in diam, which the salmon cannot move. (4) *Round boulders*: regular rocks usually greater than 102 mm (4 inches) in diam with compact fine material in between. Nearly all spawning took place over bottom of types 1, 2, and 3, and 73% over bottom of type 2. Various densities of spawning took place over each bottom type. Figures 4 and 5 show the formation of, and egg deposition on, bottom types 2 and 3 respectively.

Spawning occurred at depths ranging from 0.3 to 12.0 m, but over 90% occurred between 2 and 6 m. The 6-m contour corresponds closely with the edge of the littoral shelf. Beyond the shelf, spawning did not take place because the bottom here is frequently solid rock. On several points of land and at narrows where the slope drops rather steeply and no shelf is present, spawning occasionally occurred at depths of 9–12 m.

The slope of nearly all spawning beaches varied from 15 to 25°. Beaches with a slope of less than 15° generally have a bottom that is heavily silted and compact. These beaches were seldom utilized. At several points of land and narrows the slope approaches 40° at depths of 9–12 m. In these situations spawning was concentrated on lesser slopes and shallower depths.

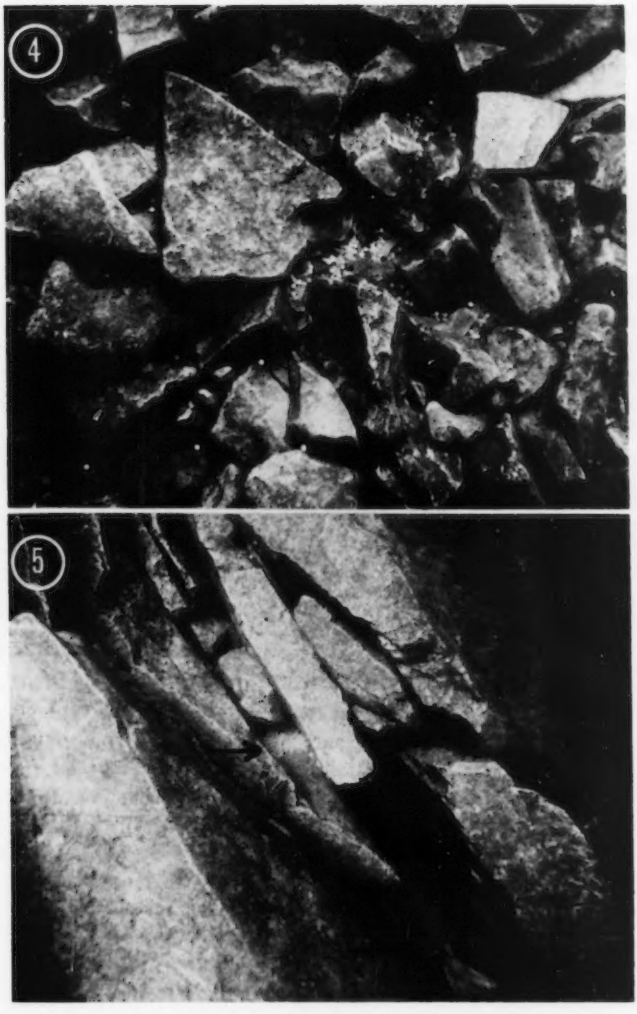

FIG. 4. Spawning bottom type 2, utilized predominantly by sockeye in 1965. Most eggs are between the rocks and are not visible.

FIG. 5. Spawning bottom type 3. Most eggs are in interstices and are not visible; one small group (*top arrow*) is visible on the ledge near the center of the photograph. Note carcass of dead sockeye salmon (*bottom arrow*) in interstice below ledge.

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SPAWNING BEHAVIOR AND SUCCESS

The distribution of transient sockeye was surveyed from airplane and boat in late July. Generally, there was a fairly even distribution of fish in shallow water along the shoreline of the entire lake. Schools of fish fringed reefs and islets. Great masses occupied the shallow, muddy backbays, such as those on Woody Island (Fig. 3). These fish were perhaps taking advantage of the relatively warmer waters in these bays, as they are unsuited for spawning because of the muddy bottom and limited water circulation. Fish were also rather evenly scattered in small schools just below the surface in the open portions of the central and eastern parts of the lake. Typically, the transient sockeye moved in an easterly direction in schools parallel to the beaches.

In mid August, as the maturation process neared its final stage, the fish moved out of the shallow backbays and collected in the spawning areas. There was no longer a directional movement of the schools, but a random milling of both sexes over the bottom (prepeak spawning stage) in depths from 2 to 6 m. Occasionally a female would dig. Close inspection from the tower and by diving in scuba revealed that, on beaches of bottom types 1, 2, and 3, only the surface silt was disturbed by this digging and the silt within the interstices remained unmoved. The digging itself appeared rather inefficient; the female began the strokes with her tail near the bottom, but her movements carried her rapidly upward so that the major portion of the action was away from the bottom.

Generally, the onset of release of eggs or milt was signaled by the first appearance of white discoloration of the fish and the formation of dense groups or clusters composed of both males and females (peak spawning stage). These groups contained from about 10 to 50 fish. The sex ratio in these groups could not be determined accurately because there was considerable local milling, especially by the males. At the bottom of the group there were always one or more active females with their vents down in the rock interstices. Males next to the active female and some males a few feet away quivered with their whole bodies. When the female passed eggs, the males next to the female and those in close proximity released milt. Local milling continued while the same or other females deposited eggs in the same area occupied by the group. This behavior was common throughout the peak spawning period in areas observed.

During spawning neither males nor females exhibited territorial defense. Fish of both sexes engaged occasionally in nipping, but this behavior appeared random and unrelated to any specific spawning area.

The eggs settled rapidly into the interstices and in most cases disappeared from sight. Where smaller bottom material filled the interstices, many eggs were visible (Fig. 4). If spawning took place over very large broken rocks (Fig. 5), some eggs rested on ledges and in interstices. In areas where spawners were dense, pockets of eggs were obvious; in some cases several gallons of eggs occupied the large depressions.

The postspawning stage was characterized by increasing extensive discoloration of the fish and by random milling over a wide area.

The stages of spawning on any one beach were not separate, but overlapped somewhat. During the spawning period usually two stages were apparent with one stage predominating.

After the postspawning stage, eggs were collected from 14 locations on the beaches and examined to determine the rate of fertilization and stage of development. Of the 3553 eggs collected, 241 eggs, or 6.8%, were infertile. The remaining eggs varied in stage of development from multicellular blastoderm to complete closure of the blastopore. The percentage of infertile eggs ranged among locations from 0, at two sites on Porcupine Island, to 15.8%, at a heavily utilized area of Porcupine Island. Only 2.8% of the eggs were infertile in collections from large rocks of Knutson Islets (Fig. 5) where spawners were also dense. Thus, the limited sampling indicated that fertilization was successful.

GENERAL DISCUSSION

On island beaches of Iliamna Lake in 1965, sockeye salmon generally spawned in groups consisting of both sexes, in exposed areas without upwelling ground water, over immovable rock, and with little territorial defense. Clearly, some of these apparently adapted features are requirements for survival of the eggs. Eggs spawned in interstices between immovable rocks in exposed areas are more susceptible to aeration by lake currents and seiches than in backbay areas of smaller substrate. Apparently spawning in dense groups is necessary for egg fertilization. Furthermore, spawning on the beaches occurs in the first 3 weeks of August, earlier than in other types of spawning grounds in the watershed. There is sufficient time for the eggs to hatch under existing water temperatures before the lake freezes (Olsen, MS, 1964), usually in December.

The spawning environment has unique advantages for the survival of eggs and fry, compared with other spawning environments in the watershed. Eggs are not subject to the scouring common in streams. Lakewater movements are not great enough to scour the rock bottom of the beaches, except perhaps in very shallow water when waves are large.

Since most eggs are deposited between immovable rocks, there is little disturbance of eggs by later spawning activity. It is likely, then, that the beaches can support much higher densities of spawners without detrimental effect than streams can. Further, eggs need not be covered with gravel by the spawners, as is necessary and many times ineffective in the slack waters of spring ponds and mainland beaches, where gravel must be moved horizontally by the fish without the aid of water current.

Finally, fry emerging from the beaches have immediate access to the large food supply in the lake, whereas fry emerging from rivers and some ponds must migrate through lake tributaries to avail themselves of this food supply.

On the other hand, there are possible hazards or disadvantages. Eggs are susceptible to predation by other fishes. Slimy sculpins, *Cottus cognatus*

Richardson, and Arctic chars, *Salvelinus alpinus* (Linnaeus), were seen gorging on salmon eggs. Examination of the stomach contents of sculpins showed that they had fed on both dead and live eggs. Sculpins are able to move along with ease among the rocks. It was not uncommon to find several under large rocks in areas where eggs were deposited. Reed (1967) considered the prickly sculpin, *Cottus asper* Richardson, a major predator on pink and chum salmon eggs in Old Tom Creek, Prince of Wales Island, Alaska. Egg loss from predation by fish perhaps is not a serious problem during a peak cycle year, such as 1965, when an enormous number of eggs are deposited. However, the high rate of survival of predator populations as a result of the good food supply could pose serious problems during low cycle years and may be a factor that accentuates the cycle.

Spawning occurs occasionally in water less than 1 m deep, especially during peak cycle years. Eggs deposited at such depths may be exposed to freezing and to ultraviolet radiation (Bell and Hoar, 1950). The lake level drops 1 m or more from late fall to spring. In lakes with the water transparency of Iliamna Lake, 50% of ultraviolet light energy penetrates to a depth of 1 m.

Other hazards to egg survival became evident in 1965. Enrichment of the water from the breakdown of salmon carcasses resulted in the rapid and luxuriant growth of algae. Although enrichment may have long-range beneficial effects (Donaldson, MS, 1967), the growth of algae may restrict water circulation to the egg surfaces and thus smother them. We might also expect some egg loss from smothering by the eggs themselves since it is likely that water circulation and gas exchange would be greatly reduced in pockets containing several gallons of eggs.

The island beach environment and spawning behavior must be favorable for the survival of sockeye because spawning has occurred here for many years, especially in peak cycle years. However, the per cent survival of eggs, extent of predation on eggs by fishes, straying of spawners to and from island areas, and racial differences among spawners in streams, ponds, mainland and island beaches are unknown.

ACKNOWLEDGMENTS

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Some Effects of Salinity and Temperature on Early Development and Survival of the English Sole (*Parophrys vetulus*)[1]

By D. F. Alderdice and C. R. Forrester

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

ABSTRACT

Eggs of the English sole were incubated at a number of salinity–temperature conditions within the ranges of 10–40‰ salinity (S) and 2–12 C. Temperatures of 2 C were lethal in all cases. Development time from fertilization to 50% hatch ranged from 3.5 days (12 C) to 11.8 days (4 C) and was shortest at salinities around 25‰ between temperatures of 6–12 C. Larvae were classified as viable (normal) or abnormal on the basis of several subjective criteria.

Response surfaces were computed and salinity–temperature conditions were estimated at which several responses were maximized, namely: larval size: 28.1‰, 7.9 C; total hatch, 25.6‰, 9.0 C; viable hatch, 25.9‰, 8.4 C. In general, optimum conditions for survival appeared to be associated with salinities and temperatures of 25–28‰ and 8–9 C. Temperatures associated with the production of 50% viable hatch or better at optimum salinity conditions (25.9‰) were calculated to be those bounded by 4.5 and 12.5 C.

Incidental observations were obtained in several cases on egg density and oxygen consumption. At 30‰ S, 6 C, eggs floated for about 93% of the incubation period and sank just prior to hatching. Salinities of neutral buoyancy of these eggs rose from 27.8‰ at fertilization to 30.8‰ at hatching. Oxygen consumption tests and calculation of rates of oxygen transfer to the eggs indicated that rates of development were independent of oxygen availability in all tests.

Several inferences are drawn from the experimental evidence regarding effects of salinity and temperature on distribution and abundance. Over the geographic range of the species, salinity would appear to have little influence on egg viability. Temperature, however, may limit abundance at the northern and southern boundaries of the range of commercial exploitation by lowering egg viability, and be lethal to eggs near the boundaries of geographic distribution.

INTRODUCTION

The English or lemon sole (*Parophrys vetulus*) is found only on the eastern side of the Pacific Ocean, where its range extends from Sebastian Vizcaino Bay, Baja California, Mexico (28°30′N lat, 115°00′W long) to Unimak Island (54°30′N lat, 164°W long) in western Alaska (Roedel, 1948; Taylor, MS, 1957, p. 43; Anon., 1964). It is one of the principal groundfish species taken by the Canadian and United States trawl fisheries in the northeastern Pacific and is exploited in the fishery from Santa Barbara, California (34°25′N lat), to northern Hecate Strait, British Columbia (54°40′N lat) (Alverson, 1960; Ketchen, 1956).

In southern British Columbia spawning of the English sole generally occurs over a 4-month period ending in late March or early April, with a maximum in early February (Ketchen, 1956).

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Eggs of the English sole are considered to be pelagic, tending to float at or near the surface (Budd, 1940). For pelagic marine eggs and larvae the interval of passive transport has been associated by many authors with a period of high and variable mortality.

Embryonic development of the species has been studied by Budd (1940) and Orsi (MS, 1965). Effects of temperature on rate of development of the egg were considered by Ketchen (1956). However, there is little evidence regarding the quantitative effects on embryonic development of variation in both salinity and temperature. The present study was designed to determine the effects of such variation on egg survival, and to relate the experimental results obtained to survival of eggs and larvae in the natural environment.

MATERIALS AND METHODS

Ripe adult English sole were captured by trawl net on January 14 and 31, 1966, at a depth of 67.7 m (37 fath) from a spawning ground off Boat Harbour in the Strait of Georgia, B.C. (49°06′N lat, 123°46′W long). Surface water temperature at Boat Harbour was approximately 7.5 C. The live fish were transported to the Biological Station, Nanaimo, B.C., where eggs were stripped and fertilized on the two occasions in seawater of 24.43 and 23.76‰ salinity (S) respectively.

Fertilized eggs were transferred to incubators held under various constant salinity and temperature conditions in a battery of identical 40-liter plastic tanks, as described by Alderdice and Velsen (1968).

Eggs used averaged 1.01 mm diam (range 0.98–1.10 mm) with a specific gravity of 1.022. Subsamples of eggs were dispensed volumetrically into the four cells of each incubation chamber. Subsamples ranged from 362 to 852 eggs with a mean of 626 eggs for all experiments. Tests were assigned to the array of tanks by a random process. The tests were conducted in 20 different salinity–temperature combinations (Table I) until completion of hatching or until it was evident that no hatch would occur.

Two sets of tests were conducted. The first set (January 14, 1966) consisted of 13 tests. Nine of these were based on a 3^2 factorial design employing salinities of 10, 20, and 30‰ and temperatures of 2, 6, and 10 C. The remaining four tests were orthogonal to the main design at 15 and 25‰ S and 4 and 8 C. All eggs died in initial stages of development in the three tests at 2 C and these tests were omitted from computation. In the second set (January 31, 1966), seven different salinity–temperature combinations were examined. These were selected so as to combine with the remaining 10 tests of the first set and restore balance to the experimental design. In addition, the 30‰ S, 10 C test was duplicated in the second set.

In the first set of tests, employing salinities up to 30‰, salt water was prepared by making natural salt water (27–29‰ S) up to 30‰ S with a synthetic seawater salt mixture[2] and diluting to the required salinity with fresh

[2]Rila Marine Mix, Rila Products, Teaneck, N.J.

TABLE I. Salinities and temperatures of incubation media.

Factor		Dates of tests (1966)	Test conditions	
			Mean ±1 standard error	
S (‰)	Temp (*C*)		S (‰)	Temp (*C*)
15	4	14/1–3/2	14.99±0.01	4.13±0.06
15	8	14/1–28/1	15.01±0.01	8.09±0.02
15	12	31/1–7/2	15.04±0.02	12.03±0.10
25	4	14/1–3/2	25.02±0.02	4.16±0.04
25	8	14/1–24/1	25.04±0.02	8.07±0.06
25	12	31/1–6/2	24.98±0.02	12.08±0.03
35	4	31/1–12/2	34.94±0.00	4.12±0.06
35	8	31/1–7/2	34.95±0.00	8.01±0.00
35	12	31/1–5/2	34.95±0.00	12.09±0.05
10	10	14/1–22/1	9.99±0.02	10.04±0.02
10	6	14/1–1/2	10.01±0.02	6.11±0.05
40	10	31/1–6/2	40.00±0.05	10.04±0.04
40	6	31/1–11/2	40.02±0.02	6.07±0.04
20	10	14/1–27/1	19.98±0.01	10.05±0.04
20	6	14/1–27/1	20.02±0.00	6.14±0.07
30	10	14/1–21/1; 31/1–7/2	29.96±0.00	10.00±0.00
30	6	14/1–28/1	29.97±0.01	6.09±0.06
10	2	14/1–24/1	10.02±0.01	2.05±0.04
20	2	14/1–24/1	19.99±0.02	2.03±0.02
30	2	14/1–24/1	29.99±0.02	2.02±0 00

water. In the second set of tests, salinities above 30‰ were prepared by making natural salt water up to 40‰ S with synthetic sea salt and diluting with fresh water as required.

Results of exposure of the eggs to the various salinity–temperature combinations were considered primarily in terms of the following responses:

1. Rate of development (%/day = $\frac{100}{T}$), where T = days from fertilization to 50% hatch of viable larvae.
2. Mean length of larvae at hatching.
3. Total hatch (A+B)/(A+B+C), where A = normal larvae, B = abnormal larvae, C = dead eggs or embryos which died prior to hatching.
4. Viable hatch, A/(A+B+C).

The hatching frequencies (numbers of normal larvae hatching) between observation periods were employed to estimate the mean hatching time as well as the incubation period, the interval between fertilization and 50% viable hatch. The distribution of hatching frequency was positively skewed in all cases in which there were sufficient larvae produced to allow examination of the distribution. On the average, 81% of the viable eggs in an incubator

hatched within one 8-hr interval. The remaining eggs which ultimately hatched did so over the following several days. Preliminary computations involved applying a square root transformation to the observation times at which larvae were counted. This adjustment normalized the distributions of hatching frequency and eliminated a pronounced association between means and variances of the raw distribution data.

Viable or normal larvae were considered to be those without curvature of the body, the most frequent type of abnormality encountered. Counts of abnormal larvae included, as well, smaller numbers of very small larvae. These appeared to hatch prematurely. Because of associated body curvature or lack of activity these larvae did not appear to have a reasonable chance to develop further under natural conditions.

For larval size, as well as hatching efficiency (percentage total hatch and viable hatch), further examination of the data was carried out by regression and eigenfunction analysis. Various transformations of the measured responses were employed initially, one being selected which maximized the variance ratios for treatments/experimental error, regression/deviations from regression, and regression/experimental error. A further requirement of the transformation accepted was that it should provide for lack of association between response means and variances.[3]

RATE OF DEVELOPMENT

Length of incubation period and rate of development of eggs held in the various salinity–temperature media are listed in Table II, along with comparable information from other sources. These data show a slight curvature in the relationship between temperature and rate of development between 4 and 13 C (Fig. 1). Development to hatching was completely inhibited at a temperature between 2 and 4 C.

The effect of salinity on the length of the incubation period at five test temperatures is illustrated in Fig. 2. In general, between temperatures of 6 and 12 C, development appeared to be most rapid at a salinity of about 25‰. At 4 C, where rate of development was slowest, incubation time appeared conversely to be shortest at approximately 25‰ S. In most cases the differences in incubation time attributed to a salinity effect are small. Nevertheless, they are considered real. Differences in numbers of newly hatched larvae observed by simultaneous examination of incubators at a given temperature repeatedly bore out this conclusion.

The effect of both salinity and temperature on incubation time is illustrated as a surface in Fig. 3. The exponential increase in incubation time with de-

[3]Transformations of Y, the original estimates of response, included $(Y + C_2)^{1/2}$, $\log_{10}(Y + C_2)$, $(Y + C_2)^{-1}$, 2 arcsin $\left[(Y + C_2)/C_1\right]^{1/2}$, and $\log_{10}\left[\frac{Y + C_2}{C_1} \Big/ \left(1 - \frac{Y + C_2}{C_1}\right)\right]$ where $C_1 = 100$, $C_2 = 0.0016$. The constant C_2 is equivalent to the smallest detectable response of 1 egg in a subsample of 600, the latter being a rounded-off approximation of the mean number of eggs in all subsamples.

TABLE II. Rates of development of English sole eggs under the various salinity–temperature test conditions. Data from other sources are appended.

Salinity (‰)	Temp (*C*)	Incubation period (*hr*)	Rate of development (%/*day*)	Data from other sources
15	4	276.26	8.7	
15	8	140.30	17.1	
15	12	87.25	27.5	
25	4	281.13	8.5	
25	8	138.06	17.4	
25	12	84.20	28.5	
35	4	264.26	9.1	
35	8	147.89	16.2	
35	12	87.39	27.4	
10	10	121.33	19.8	
10	6	201.16	11.9	
40	10	108.64	22.1	
40	6	196.50	12.2	
20	10	108.41	22.2	
20	6	181.95	13.2	
30	10	110.02, 108.02[a]	22.0	
30	6	184.31	13.0	
–	12.9	90	26.7	Ketchen, pers. comm.; see also Ketchen, 1956, Fig. 20
–	10.3	120	20.0	
–	8.3	140	17.1	
–	7.7	145	16.5	
–	7.1	175	13.1	
–	10.6	101[b]	23.8	Orsi, 1965
–	13.0	90	26.7	Budd, 1940

[a]January 14 and 31 tests, respectively.
[b]Period of development estimated by present authors from original data as period to beginning of hatch + square root of hatching interval.

creasing temperature is indicative of the obviously greater influence of temperature, compared with that of salinity, on rate of development.

SIZE OF LARVAE

To compare larval sizes in the various tests, measurements of larvae were made at times approximating the midpoints of hatching frequency. From previous studies (Forrester and Alderdice, 1966) it was apparent that variations in time of measurement with respect to hatching frequency could bias estimates of mean larval size. To reduce such bias an adjustment was made to the estimates of mean larval size as measured, based on the number of hours elapsed between the computed time of 50% hatch and the time at which larval meas-

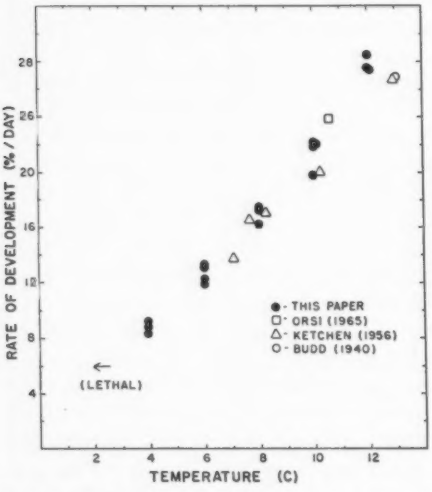

FIG. 1. Influence of temperature on rate of development from fertilization to 50% hatch for English sole eggs. The several points at each temperature for the data (solid circles) are at the various salinities of the factorial design employed (see Table II).

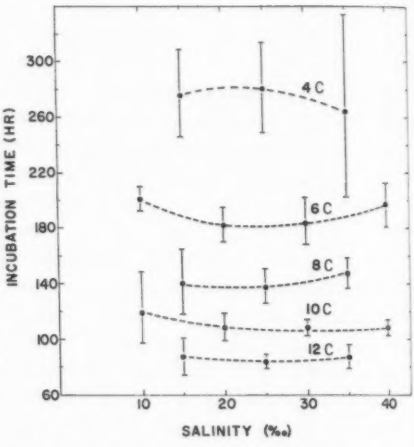

FIG. 2. Influence of salinity on the time to 50% hatch of English sole eggs held at five constant temperatures from 4 to 12 C. Individual tests are shown as mean incubation times within the ranges of ±2 standard deviations (hr).

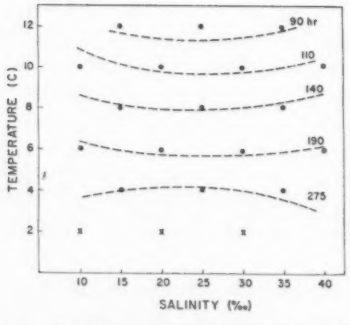

FIG. 3. The effect of salinity and temperature on incubation time of English sole eggs. Isopleths for equal hatching time have been approximated. Salinity–temperature conditions of the various tests are indicated by the array of points; at 2 C all eggs died.

urements were made.[4] The original and adjusted larval lengths are listed in Table III. Although the adjustment is one of simple proportionality, it has the effect of making estimates of larval length approach more closely those which would have been obtained had each sample been measured at the same interval in the various distributions of hatching frequency.

TABLE III. Measurements of larvae from the various salinity–temperature tests. Observed mean lengths are listed, as well as adjusted mean lengths at the time of 50% hatch. The adjusted means are based on a correction for growth occurring in the interval between time of 50% hatch and time of measurement (see text).

Salinity (‰)	Temp (C)	Mean hatching time, t_0 (hr)	Time of measurement, t_1 (hr)	Mean length, L (mm)	Adjusted mean length, $L \times t_0/t_1$ (mm)
15	4	276.3	281.5	2.61	2.56(2.55)[b]
15	8	140.3	149.1	2.93	2.76
15	12	87.3	93.1	2.81	2.63
25	4	281.1	283.0	2.89	2.86(2.88)[b]
25	8	138.1	141.5	2.98	2.92(2.95)[b]
25	12	84.2	91.2	2.99	2.77
35	4	264.3	–	–[a]	–
35	8	147.9	148.8	2.80	2.77(2.78)[b]
35	12	87.4	96.5	2.95	2.71
10	10	121.3	–	–[a]	–
10	6	201.2	–	–[a]	–
40	10	108.6	115.8	2.98	2.79
40	6	196.5	195.8	2.70	2.70
20	10	108.4	114.6	2.89	2.75
20	6	182.0	187.3	2.86	2.78
30	10	108.0	114.6	2.96	2.79
30	6	184.3	186.5	2.87	2.84

[a]Too few normal larvae were obtained to provide an estimate of mean length.
[b]Graphic estimate of mean larval length (see footnote 4 in text).

The adjusted mean larval lengths are listed on a salinity–temperature grid in Table IV. Largest larvae were obtained from eggs incubated at 25‰ S and 8 C.

The number of larvae available for measurement varied among subsamples and between tests. Because of the small numbers of larvae available in some tests, subsample measurements were combined into one single sample for each test condition. The resulting loss of information on subsample effects eliminated the treatment × subsample interaction normally used as a measure

[4]In four of the tests, larvae were measured close to as well as at intervals after the time of 50% hatch. The relationships between time and larval length so obtained were used graphically to check on the expected size of larvae at the time of 50% hatch. In the four cases the differences between the estimates of larval length, obtained either proportionally or graphically, averaged 0.02 mm (see Table III). The proportionally adjusted larval lengths are accepted therefore as reasonable estimates of mean larval length at the time of 50% hatch in each test condition.

TABLE IV. Estimates of mean larval lengths (mm) at the time of 50% hatch.

Salinity (‰)	Temp (*C*)				
	4	6	8	10	12
10		–		–	
15	2.56		2.76		2.63
20		2.78		2.75	
25	2.86		2.92		2.77
30		2.84		2.79	
35	–		2.77		2.71
40		2.70		2.79	

of experimental error. Further analysis of the relationship of larval size to incubation levels of salinity and temperature therefore was based on components of variance associated with regression and deviations from regression.

Table IV suggests that the relationship could be examined through standard regression methods, assuming that a second order polynomial would provide an adequate fit to the data. Preliminary computer analyses indicated that larval size would best be considered in terms of Y, the original mean sample lengths. Hence:

$$Y = b_0x_0 + b_1x_1 + b_2x_2 + b_{11}x_1^2 + b_{22}x_2^2 + b_{12}x_1x_2$$

where x_1, x_2 = coded levels of salinity and temperature[5] and Y = larval length (mm).

Lacking an error term, the total variance was divided into two components; one is associated with regression on x_1, x_2, x_1^2, x_2^2 and x_1x_2, the other a component unexplained by regression on these factors. The 5 degrees of freedom associated with regression were further subdivided into components of variance associated with linear, quadratic, and interaction terms.

Source of variance	SS	df	MS	F
Regression on all factors	0.07671	5	0.01534	4.28*
Linear	0.01772	2	0.00886	2.47NS
Quadratic	0.05853	2	0.02977	8.32*
Interaction	0.00050	1	0.00050	0.14NS
Deviations from regression	0.03078	8	0.00385	
Total	0.10749	13		

*F.95 (5, 8) = 3.69; *F.95 (2, 8) = 4.46; *F.95 (1, 8) = 5.32.

[5] Coded factor	Factor	Coded level −1	0	1	Base level	Unit
x_1	S(‰)	15	25	35	25	10
x_2	Temp(*C*)	4	8	12	8	4

x = (factor – base level)/unit (e.g.) for 35‰ S; x_1 = (35–25)/10 = 1

The analysis suggests that regression explains a statistically significant portion of the total variance. The effects of salinity and temperature could be designated by an equation involving only quadratic terms. In order to describe the regression or response surface with respect to salinity and temperature, however, all terms were retained as the best available estimates. Nevertheless, it is recognized that the linear and interaction effects of salinity and temperature are not statistically significant.

The fitted equation is

$$Y = 2.86261 + 0.05818x_1 - 0.00715x_2 - 0.09175x_1^2 - 0.08978x_2^2 - 0.00881x_1x_2.$$

For convenience in describing the form of the response surface in terms of salinity and temperature the polynomial equation was reduced to its equivalent canonical form (Box, 1954, 1956) (see Forrester and Alderdice, 1966).

Hence:

$$Y - Y_s = \lambda_{11}X_2^1 + \lambda_{22}X_2^2$$

where Y_s = larval size estimated at the centre of the response surface S; λ_{ii} = the rate at which the response changes in the direction of the X_i axes of the response surface.

By calculation, the equation of the response surface is

$$Y - 2.87174 = -0.0953X_1^2 - 0.0863X_2^2.$$

Y_s, the computed maximum larval size, is found (Box, 1956) at the centre of the response surface where in coded units $x_{1s} = 0.30919$, $x_{2s} = -0.03632$. Converting to original units (see footnote 6) the centre of the surface is found at or in the vicinity of 28.1‰ S and 7.9 C. The regression of larval size on salinity and temperature is illustrated in Fig. 4. Maximum larval length was calculated to occur at approximately 28.1‰ S, 7.9 C. In terms of scale, the effect of a temperature change of 1 degree C on larval length is approximately equal to that produced by a change of 2.5 parts per mille S.

HATCHING EFFICIENCY

Table V lists the percentages of eggs producing live larvae (total hatch) whether normal or abnormal, as well as the percentages of eggs which produced viable or normal larvae (viable hatch).[6]

Both total and viable hatches, as percentages, were highest in the test at 25‰ S, 8 C.

At 4 C, total hatch was low in all salinities (15, 25, 35‰) and the rate of development and the state reached were irregular. Most embryos developed in approximately 110 hr to a stage in which the germ ring was overgrowing

[6]Other obvious but correlated estimates may be obtained from Table V. Hence 100 – (total hatch) = % egg mortality; (total hatch) – (viable hatch) = % abnormal larvae; (total hatch)/(viable hatch) = % viable larvae.

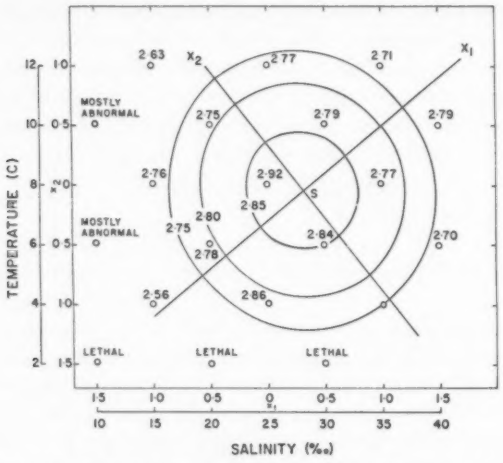

FIG. 4. Calculated regression surface showing the relationship between salinity and temperature levels and larval size in millimeters. Isopleths are shown at 2.75, 2.80, and 2.85 mm. The X_1-, X_2- axes with centre S are the geometric axes of the regression surface. Salinity and temperature levels calculated at S are 28.1‰ S and 7.9 C. The array of points in the figure locates the conditions of the experimental trials; the values in the array represent the adjusted larval lengths for the various trials.

the yolk. However, many eggs failed to reach the stage of blastopore closure. Death of eggs was generally concurrent with collapse and partial invagination of the chorion, suggestive of osmoregulatory failure.

As noted earlier a temperature of 2 C was lethal to the sole eggs over a 10–30‰ salinity range. In these tests development was arrested at the blastodermal cap stage after approximately 80 hr incubation. Subsequently the chorion collapsed in the same manner as those at 4 C and the eggs died. Observations at 2 and 4 C suggest that mortality may be caused primarily by temperature per se or by its effect on osmotic control.

With respect to salinity an increasing tendency was evident for larvae to develop abnormally above and below the range of 25–30‰. In the 20 and 30‰ S tests at 6 and 10 C larval production was relatively high. However, in the 10‰ S tests at 6 and 10 C viable hatch was low, suggesting that salinities of that order produce an osmoregulation problem more or less independent of temperature level. In both cases (10‰ S, 6 and 10 C) evidence of abnormal development was apparent from the time of formation of the blastodermal cap. Although development continued in the majority of eggs, many died unhatched at an advanced stage of development. Larvae which did hatch were unable to shed the chorion, were markedly inactive, or had gross curvature of the body.

TABLE V. Incubation of English sole eggs at 17 salinity–temperature (S, T) combinations. Total hatch refers to the percentage of eggs which hatched. Viable hatch refers to the percentage of eggs which produced larvae of normal appearance.

Factor levels		Total no. of eggs Subsample				Total hatch, % Subsample					Viable hatch, % Subsample				
S (‰)	Temp(*C*)	1	2	3	4	1	2	3	4	Mean	1	2	3	4	Mean
15	4	666	724	764	723	35.44	28.04	23.95	29.32	29.19	6.31	8.15	3.93	7.33	6.43
15	8	656	747	746	703	91.46	93.31	82.44	91.18	89.60	90.24	75.77	64.08	81.79	77.97
15	12	566	603	560	394	71.91	56.88	65.18	76.65	67.66	17.31	7.63	26.61	15.99	16.87
25	4	717	782	852	590	28.31	22.63	18.19	23.39	23.13	7.11	10.61	3.29	7.12	7.03
25	8	621	640	754	570	95.17	88.13	94.69	93.33	92.83	95.17	86.56	94.03	85.26	90.26
25	12	622	645	608	532	76.37	72.09	83.22	78.01	77.42	67.52	48.84	66.61	67.11	62.11
35	4	738	655	742	743	0.14	0.46	1.35	0.40	0.59	0.14	0.15	0.40	0.13	0.21
35	8	616	467	484	606	85.39	89.72	84.71	61.72	80.74	53.25	84.37	79.13	31.68	62.02
35	12	362	478	590	459	75.14	73.64	66.44	83.22	74.61	19.06	11.51	13.22	18.52	15.58
10	10	681	710	611	700	44.49	46.34	42.88	43.00	44.18	0.00	0.00	0.00	0.00	0.00
10	6	757	681	647	801	36.59	35.24	40.80	36.83	37.37	0.00	0.88	0.15	1.00	0.51
40	10	450	553	564	604	86.00	70.34	68.79	52.65	69.45	23.33	29.48	60.46	30.13	35.85
40	6	662	542	527	591	41.69	45.57	47.06	25.21	39.88	20.24	19.00	24.67	13.54	19.36
20	10	391	650	603	548	89.77	86.00	87.40	86.86	87.51	87.98	81.54	82.75	83.03	83.83
20	6	643	671	497	771	90.98	92.40	87.93	86.51	89.46	89.42	76.45	78.27	83.40	81.89
30	10	532	605	563	559	91.07	89.01	90.05	90.96	90.27	85.24	85.37	65.72	74.31	77.66
30	6	666	704	656	723	84.53	85.23	85.67	85.06	85.12	72.07	83.10	84.15	76.63	78.99

The more prominent anomalies and qualitative differences in development are outlined below. A number of these are illustrated in Fig. 5 in approximate relation to associated salinity and temperature levels.

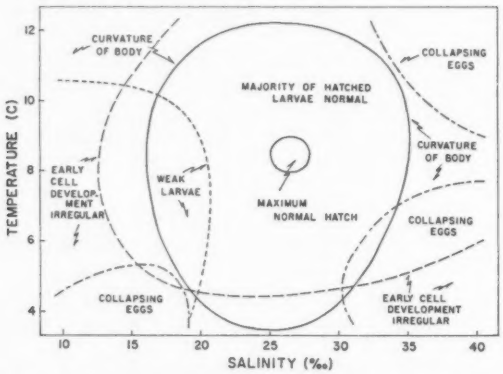

FIG. 5. Approximate areas over the salinity–temperature range examined in which various qualitative differences in development were observed.

Early cell development irregular — usually a gross irregularity of cell sizes in the blastodermal cap. At salinities below 20‰ for all temperatures from 2 to 12 C, and at all salinities at temperatures below 6 C.

Collapsing eggs — invagination of the chorion. At salinities above 30‰, predominately at 6 and 12 C, and below 20‰ S at 4 C.

Death of embryo at early developmental stage — generally at the stage of blastodermal cap formation. At all salinities (10, 20, 30‰) at 2 C.

Death at advanced stage of egg development — from the stage of blastopore closure to near full-term. At salinities from 15 to 35‰ at 4 C, at 10‰ at 6 C.

Weak larvae — larvae hatching either head- or tail-first but unable to shed chorion, inactive. Generally below 20‰ S from 4 to 10 C.

Pronounced curvature of body. At salinities below 20‰ and above 30‰. Most prominent below 20‰ at 6–12 C and above 30‰ at 4–8 C.

Normal larvae. Highest numbers obtained at 20–30‰ S and 8–10 C.

Further analyses of the effects of salinity and temperature on hatching efficiency were restricted to a consideration of total hatch and viable hatch. Response surfaces were developed in each case in the same manner as that employed for description of larval size.

Analysis of variance of the data (Table V) was conducted on the basis of the dependent variable transformations listed earlier (see footnote 4). In both sets of data concerned with hatching efficiency equalization of variance for the individual tests was most closely approximated by transformation of percentage response (Y) to

$$U = 2 \arcsin \sqrt{\frac{Y + C_2}{C_1}}.$$

TOTAL HATCH

The analysis below, based on the data of Table V, indicates that subsample effects concerned with differences between responses in the four incubation baskets of individual incubators can be ignored, and that treatment effects are highly significant in comparison with the pooled error term.

Source of variance	SS	df	MS	F
Treatments	30.003	16	1.875	84.09**
Residual (pooled)	1.138	51	0.022	
Subsample effect	0.106	3	0.035	1.64^{NS}
Treatment × subsample interaction	1.032	48	0.022	
Total	31.141	67		

**F.99 (16, 51) = 2.38; *F.95 (3, 48) = 2.80.

In considering total hatch it was again assumed that a second order polynomial would provide an adequate fit to the data. Analysis proceeded as conducted for larval size, but with Y = U. The treatment variance was partitioned into a component associated with total regression on x_1, x_2, x_1^2, x_2^2 and x_1x_2, and a component not explained by regression on these factors.

Source of variance	SS	df	MS	F
Treatments	30.003	16		
Total regression	27.086	5	5.417	246.23**
Deviations from regression	2.917	11	0.265	12.05**
Residual (pooled)	1.138	51	0.022	
Total	31.141	67		

**F.99 (5, 51) = 3.40; **F.99 (11, 51) = 2.62.

The statistical significance of the component associated with deviations from regression is interpreted thus: although regression has accounted for a major portion of the variance associated with treatment effects, a significantly large portion of the treatment variance remains unaccounted for with respect to the fitting procedure utilized. Nevertheless, the regression relationship employed remains a useful one although in all likelihood an improved fit could be obtained using a more sophisticated polynomial.

Partitioning of treatment variance was completed by stepwise addition of all linear, quadratic, and interaction terms.

Source of variance	SS	df	MS	F
Regression	27.086	5		
Linear	9.291	2	4.641	17.51**
Quadratic	16.314	2	8.157	30.78**
Interaction	1.482	1	1.482	5.59*
Deviations from regression	2.917	11	0.265	
Total	30.003	16		

**F.99 (2, 11) = 7.20; *F.95 (1, 11) = 4.84.

The fitted equation is

$$U = 2.752 - 0.014x_1 + 0.538x_2 - 0.408x_1^2 - 1.058x_2^2 + 0.239x_1x_2.$$

Expressing the polynomial as its canonical equivalent

$$U - 2.82175 = -1.0792X_1^2 - 0.3869X_2^2.$$

The response surface for percentage total hatch is illustrated in Fig. 6. The surface is elliptical, with maximum total hatch estimated at 97% calculated to occur at or in the vicinity of 25.59‰ S and 9.04 C. Interaction between

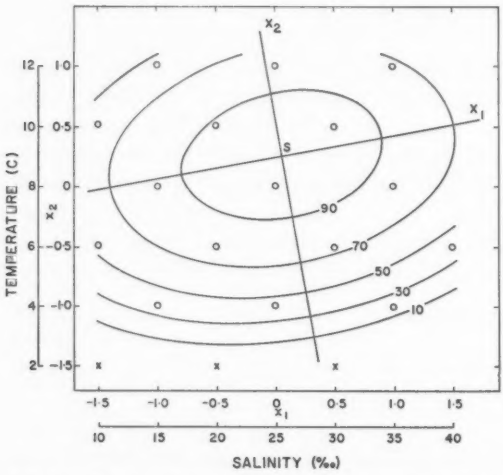

FIG. 6. Regression surface showing the relationship between salinity and temperature and per cent total hatch. Isopleths are shown at 10, 30, 50, 70, and 90% total hatch. The X_1- X_2-axes with centre S are the geometric axes of the regression surface. Salinity and temperature levels calculated at S are 25.59‰ S and 9.04 C. The array of points in the figure locates the conditions of the experimental trials. At 2 C all eggs died.

salinity and temperature is shown (Fig. 6) in the lack of coplanarity between the major axes of the response surface (X_1, X_2) and those of measurement (x_1, x_2). The interaction effect is demonstrated by calculation of the temperatures at which maximum total hatch would be expected over a range of salinities:

S(‰)	15	20	25	30	35
Temp (C)	8.6	8.8	9.0	9.2	9.5

Salinity and temperature did not have equal effects on total hatch. The effect of a change of 1 degree C on total hatch is approximately equal to that of a change of 4 parts per mille S.

Viable Hatch

Further analysis of the data was conducted on the numbers of normal larvae obtained under the various salinity–temperature conditions. The ecological implications of salinity–temperature effects on larval production might be judged more realistically in terms of normal larvae, or larvae deemed to be viable under natural conditions. Analysis of percentage viable hatch was carried out in the same manner as that in the previous section.

Source of variance	SS	df	MS	F
Treatments	49.700	16	3.106	72.12**
Residual (pooled)	2.197	51	0.043	
Subsample effect	0.080	3	0.027	0.61NS
Treatment × subsample interaction	2.116	48	0.044	
Total	51.897	67		

**F.99 (16, 51) = 2.38; *F.95 (3, 48) = 2.80.

Again, treatment effects were partitioned with respect to regression.

Source of variance	SS	df	MS	F
Treatments	49.700	16		
Total regression	44.585	5	8.917	207.37**
Deviations from regression	5.115	11	0.465	10.81**
Residual (pooled)	2.197	51	0.043	
Total	51.897	67		

**F.99 (5, 51) = 3.40; **F.99 (11, 51) = 2.62.

Finally, the variance associated with total regression was partitioned into linear, quadratic, and interaction sets.

Source of variance	SS	df	MS	F
Regression	44.585	5		
Linear	4.102	2	2.051	4.41*
Quadratic	40.196	2	20.098	43.22**
Interaction	0.288	1	0.288	0.62NS
Deviations from regression	5.115	11	0.465	
Total	49.700	16		

*F.95 (2, 11) = 3.98; **F.99 (2, 11) = 4.84.

Analysis again indicates that the data may be characterized as a quadratic regression surface, although a better fit might be obtained if the design included higher order terms. A significant portion of variance associated with regression is explained by linear and quadratic effects, but not by the interaction term. Again, however, all terms in the polynomial were retained as the best available estimates in order to fit the response surface.

The fitted equation is

$$U = 2.723 + 0.129x_1 + 0.308x_2 - 0.764x_1^2 - 1.435x_2^2 + 0.105x_1x_2.$$

Reduced to canonical form

$$U - 2.74611 = - 1.4391X_1^2 - 0.7596X_2^2.$$

The response surface for percentage viable hatch is illustrated in Fig. 7. Maximum viable hatch of about 96% was calculated to occur at or near 25.92‰ S and 8.44 C. The effect of a change of 1 degree C is approximately equivalent in effect on viable hatch to a change of 3.5 parts per mille S.

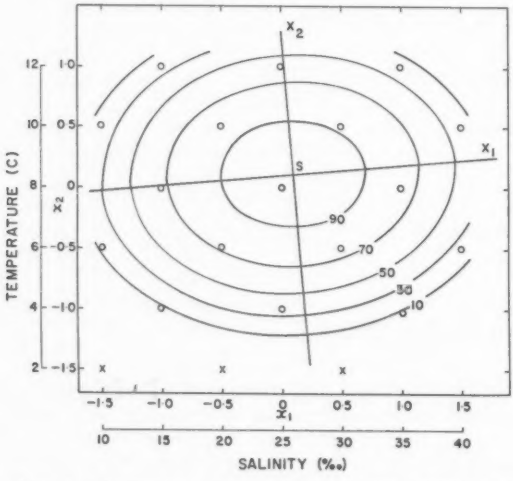

FIG. 7. Regression surface showing the relationship between salinity and temperature and per cent viable hatch. Salinity and temperature levels calculated at S are 25.92‰ S and 8.44 C. (For further details see Fig. 6.)

ADDITIONAL OBSERVATIONS

Incidental observations pertinent to these studies were made with respect to buoyancy and oxygen consumption of English sole eggs.

Buoyancy

The length of the incubation period of pelagic eggs will determine to a large extent the distance over which eggs might be transported by wind- and tide-driven currents in the interval between fertilization and hatching. Orsi (MS, 1965) noted that English sole eggs sank to the bottom of experimental containers just prior to hatching. In the present study eggs floated in the 30, 35, and 40‰ S tests, and sank in the lower (10, 15, 20, 25‰) salinities. At 30‰ S the eggs began to sink just prior to hatching. At 30‰ S, 10 C the inception of sinking was not observed, although it occurred in the interval between 50‰ hatch (108.0 hr incubation) and the preceding observation (100.6 hr). At 30‰ S, 6 C eggs began sinking at 172.6 hr. In both of these cases the eggs floated for approximately 93% of the incubation period.

Further trials were performed in order to examine the assumed changes in density of the developing eggs. Samples of eggs were withdrawn from a parallel test at 30‰ S, 6 C and placed in a series of graded salinities at intervals throughout the incubation period. Salinities associated with neutral buoyancy were established and checked by titration. The following values were obtained:

Incubation period (*hr*)	Neutral buoyancy (‰ *S*)	Remarks
0	27.81	— after fertilization in 23.76‰ S; SG of eggs, 1.0210 at 14.6 C
22.8	28.00–28.31	
44.1	28.34	
67.6	28.59	
96.6	28.97	
115.6	28.49	
139.1	28.51	
173.1	29.94	— eggs beginning to sink (172.6 hr)
187.1	30.78	— hatching in progress (mean – 184.3 hr)
187.1	30.09	— neutral buoyancy of larvae

The results indicate that salinity of neutral buoyancy was fairly constant between about 28 and 28.5‰ S, from fertilization until the 6th day of incubation. In the following 48 hr a rapid rise in egg density occurred which terminated with hatching. The data suggest that English sole eggs would sink in waters of 27–30‰ S, depending on their stage of development, and that above 30‰ S the eggs would remain floating throughout the incubation period.

Oxygen Consumption

Earlier studies (Forrester and Alderdice, 1966) pointed to the necessity for maintaining adequate dissolved oxygen levels when examining the effects of other physical factors on egg development. Incubation apparatus was built for the present study to provide perfusion velocities in the order of 2000 cm/hr, rates suggested by reference to studies of salmonid egg development (Wickett, 1954; Silver et al., 1963; Shumway et al., 1964).

Estimates of oxygen consumption were obtained for English sole eggs by cycling the perfusion medium through a standard egg incubator used as a closed respirometer. Although these estimates are rather gross, they do allow consideration of mass transfer of oxygen to the eggs and verification of flow rates of the perfusion medium at which respiration rate would be independent of the rate of transport of dissolved oxygen past the egg capsule (Daykin, 1965).

Oxygen consumption was measured in the 20‰ S, 6 C test 24 hr prior to the beginning of hatching. It was assumed on the basis of parallel evidence on the eggs of other species that oxygen uptake would be near a maximum at that time. The number of eggs which subsequently hatched in this test (2309) was taken as an estimate of the number of eggs respiring at the time of determination of oxygen uptake. A drawdown of 0.73 mg/liter of dissolved oxygen from an initial level of 10.92 mg/liter was obtained in 120 min. Oxygen consumption therefore was estimated at 7.633×10^{-8} mg/sec per embryo, or 0.560 mg/g of embryo per hr.[7] Under the test conditions for water holding 10.92 mg/liter dissolved oxygen, a perfusion velocity of about 10 cm/hr was calculated to be sufficient for the English sole eggs.[8] Although the calculated rate would vary over the test conditions examined, the perfusion velocity employed (1960–2200 cm/hr) was more than ample to avoid respiratory dependence of rate of development on rate of oxygen delivery to the eggs.

DISCUSSION

Experimental Observations

Salinity, temperature, and dissolved oxygen are factors which may have a major influence on aspects of development of teleost eggs (Kinne, 1964). For the pelagic eggs of the English sole, however, oxygen is not likely to be a critical factor in the natural environment. In this study emphasis was placed on aspects of development and survival as influenced by salinity and temperature only. Experimental conditions were set up so that oxygen could be maintained at sufficiently high concentrations to provide independence of response over levels of that factor. The hatch attained (Table V) and estimates of mass transfer of dissolved oxygen both indicate that independence of rate of oxygen transport was achieved.

The relationship found between temperature and rate of development (to time of 50% hatch) for the present experiments is in reasonable agreement with results of other studies on *P. vetulus* with which they are compared (Fig. 1).

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[7]Embryo weight estimated in terms of volume × density, assuming total egg volume is living material (capsule thickness, 0.0014 cm (Budd, 1940); egg diameter, 1.00 mm; S.G. of eggs 1.021).

[8]Oxygen concentration in the boundary layer at the egg surface was calculated as 4.89 mg/liter. Velocity required to maintain 4.89 mg/liter at the egg surface, 3.0 mg/liter under the capsule (see Daykin, 1965, p. 167), with ambient dissolved oxygen at 10.92 mg/liter was calculated as 8.8 cm/hr or approximately 10 cm/hr (20‰ S, 6 C). Mass transfer coefficient estimated as 4.032×10^{-4} cm²/sec; diffusion coefficient for oxygen in water estimated as 1.313×10^{-5} cm²/sec; diffusion constant for oxygen through the capsule, 1.8×10^{-4} cm²/sec (Daykin, 1965, p. 168).

The relationship between salinity, temperature, and rate of development suggests that salinities below and above approximately 25‰ may "delay" development between temperatures of 6 to 12 C. It is considered reasonable to describe the salinity effect on the incubation period in this manner. The length of the incubation period shows an association both with the relationship between salinity and hatching success and with that between salinity and larval size at hatching. Both latter responses to salinity are maximized at the same intermediate salinities which minimize the developmental time at a given temperature. It is speculated that these relationships are dependent on conditions which minimize the energy required of the embryo in maintaining osmotic equilibrium with its environment. In a comparable case maximum percentage hatch of Atlantic herring (*Clupea harengus*) eggs may be estimated as occurring at a salinity near or somewhat above 20‰ (see Holliday and Blaxter, 1960, Fig. 3). The same species appears to require least osmotic regulation at or somewhat above 17.5‰ S (see Holliday and Jones, 1965, Fig. 3).

Conversely, however, low and high salinities (15, 35‰) at 4 C appear to "accelerate" development (or the hatching process) even though larval production is much reduced at those levels. In this case the combination of an osmotic stress and a temperature approaching the lower incipient lethal level is assumed to impose a major obstacle to continued development. Such "accelerated" hatching under adverse salinity–temperature conditions perhaps may parallel the results of Buznikov (1957), who found that premature hatching of eggs subjected to hypoxia was associated with early production of hatching enzyme.

The response surface techniques employed to explore the effects of salinity and temperature on development lead primarily to an appreciation of the general relationship between the two factors investigated and hatching efficiency. Because of the empirical nature of the regression techniques employed, however, the surfaces constructed should be considered as approximations rather than exact descriptions of these relationships.

In terms of total hatch, incubating English sole eggs are tolerant of a wide range of salinities. Ninety per cent total hatch was calculated to occur within a range of 17 parts per mille S (17–34‰) and 4 degrees C (7–11 C) around the response centre (Fig. 6). Thus, a change of 1 degree C is about equivalent in effect on total hatch to a change of 4 parts per mille S, and variations in temperature have a greater potential effect on viability than variations in salinity. The appreciable interaction effect of salinity and temperature on total hatch is such that, with increases (or decreases) in salinity, maximum survival is maintained only with coupled increases (or decreases) in temperature. This interaction is equivalent to the "beneficial effects of low/low–high/high combinations" of factors reported elsewhere (Kinne, 1964). In general such "low–high" phenomena are interaction effects which describe the

direction and magnitude of change in level of one factor required to maintain a maximum response at a particular level of another factor.[9]

In terms of viable hatch, English sole eggs show a more restricted range of salinity tolerance. In general, 90% viable hatch was calculated to occur within a range of 12‰ S (20–32‰) and 3.5 C (6.5–10 C) around the response centre (Fig. 7). Similarly, 50% viable hatch occurs within a range of 27‰ S (12–39‰) and 8.0 C (4.5–12.5 C) around the centre. Temperature remains the more significant of the two factors in its effect on larval production. No statistical significance can be attached to the interaction in this case. However, the slight rotation of surface axes in Fig. 7 indicates the possible existence of a low/low–high/high interaction similar to but smaller than that found for total hatch.

Combined Effects of Salinity and Temperature on Egg Development

It is apparent that eggs of *P. vetulus* are euryhaline and that their ability to develop successfully over a range of salinities is restricted by temperature (Fig. 6, 7). Calculated salinity–temperature conditions which appear to optimize the developmental processes considered are:

Characteristic	S (‰)		Temp (*C*)	
	Mean	Range[a]	Mean	Range[a]
Minimum incubation period, 6–12 C		23.0–27.0		
Maximum larval length	28.1		7.9	
Total hatch	25.6	25.2–26.2	9.0	8.9–9.4
Viable hatch	25.9	24.9–26.9	8.4	7.9–8.5

[a]Based on variation of centre points computed for individual replicates.

The maximum estimated ranges of variation in the computed centre points for total hatch and viable hatch are approximately 2‰ S and 0.5 C. No comparable estimates of variation are available for larval length.

In general, larval length, total hatch, and viable hatch would appear to reach maximum values in the range of 25–28‰ S and 8–9 C. These maxima are also in the same approximate salinity range at which shortest incubation times occurred (for temperatures between 6 and 12 C).

Ecological Implications

It is postulated here that developmental processes of the teleost egg are optimized within a fairly restricted range of physical environmental conditions; when optimized they should culminate in a maximum potential for development and survival. The basic metabolic costs of embryonic development

[9]The interaction may be visualized in Fig. 6 as the rotation of the X_1-, X_2-axes of the surface about its centre (i.e. the axes of the surface and those of measurement are not coplanar).

are assumed to be those associated with provision of energy for maintenance, growth, and physical activity of the embryo prior to hatching. The inference follows that environmental conditions which allow a maximum ratio of energy distribution in favour of embryonal growth, while satisfying requirements for maintenance and physical activity, will produce a maximum number of normal larvae of maximum size in a minimum period of incubation. Such conditions should be particularly advantageous to pelagic eggs subject to the vagaries of drifting in a potentially hostile environment and should result in "maximum survival potential" of the egg, at least up to the time of hatching.

Under natural conditions, however, maximum survival potential might be modified by factors other than salinity and temperature. These could include environmental entities such as dissolved oxygen and its availability, biotic factors such as type and extent of predation, or physical phenomena such as rate and direction of drift of pelagic eggs and presence or absence of suitable substrate for metamorphosed post-larvae. It follows that experimentally derived estimates of optimum conditions for egg development may not agree with those found to be associated with maximum production of the species in the natural environment. Obviously, such differences would suggest that other factors beyond those examined may contribute to the complex of circumstances determining either egg survival or year-class strength.

It is instructive to consider how the general distribution of English sole in nature compares with a hypothetical distribution based on the following simplified assumptions: (i) that annual abundance or year-class strength is determined largely by environmental conditions during the egg stage; (ii) that the egg stage everywhere is found largely during the month of February; (iii) that temperature is of overriding importance; (iv) that temperatures which produce at least a 50% viable hatch constitute the requirements for existence of a population of sufficient magnitude to attract a commercial fishery; and (v) that temperatures approximating 0% hatch experimentally will define the limits of geographic distribution of the species.

The assumptions outlined above are based on the following observations. Surface salinities for February show considerable variation throughout the range of the English sole, with low mean values occurring in areas affected by freshwater runoff (Anon., 1962; Hollister, MS, 1964). Excluding data from some of the hydrographic stations considered atypical of open coastline conditions (e.g. Fraser River, B.C.; Columbia River estuary, San Francisco Bay), salinities from 42 stations from Dutch Harbor, Alaska, south to Ensenada, Mexico (main geographic locations are shown in Fig. 8) are summarized as follows for the month of February. Monthly mean surface salinities range from 25.3 to 33.7‰, there being a trend toward higher salinities (and temperatures) in the southern part of the range. Mean maximum salinities (means of monthly maxima) range from 29.5 to 34.1‰, and mean minima from 21.7 to 33.5‰ (Anon., 1962). In the region south of Ensenada to Sebastian Vizcaino Bay, Mexico (most southerly record of the English sole), salinities in the month of February (10-m depths, 1950–59) range between 33.2 and 34.3‰ (Anon.,

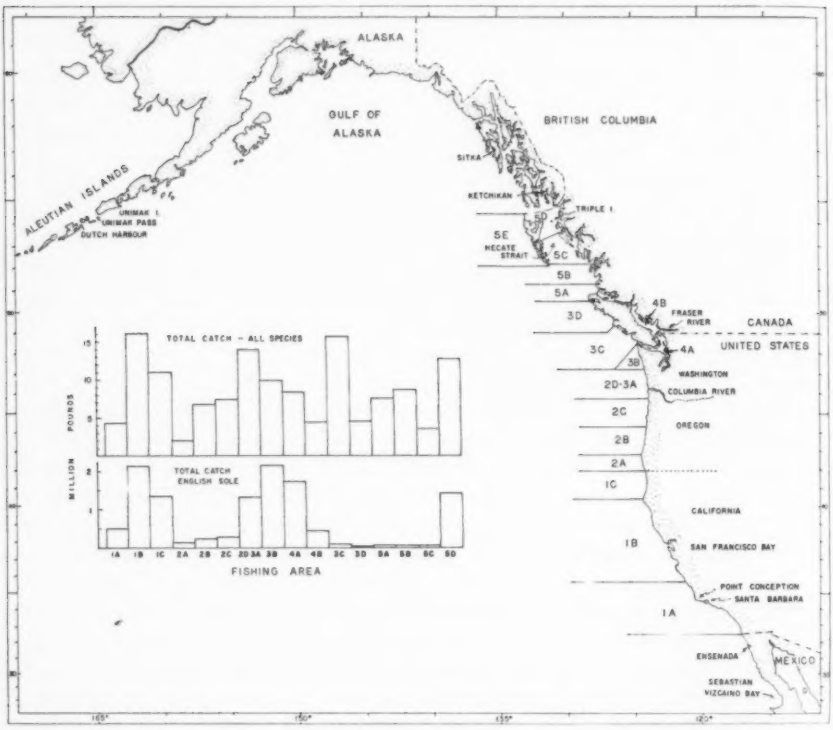

FIG. 8. Range and commercial catch of English sole. The species is recorded from Unimak Pass, Alaska, to Sebastian Vizcaino Bay, Mexico. The coastal divisions represent statistical areas of the North American fishery; their range approximates the region of commercial abundance (Hecate Strait, B.C., to Santa Barbara, Calif.). Low yield of English sole in some fishing areas is reflected by the difference between the catches for all species and those for English sole only (inset).

1963). Over the range of the English sole the lower and upper limits of coastline salinity appear to be near 20 and 35‰.

No evidence is available on levels of salinity which might limit development of English sole eggs in the natural environment. On the basis of the present experimental evidence, however, salinities in the range of 20–32‰ are entirely encompassed by the 90‰ isopleth of viable hatch at an optimum temperature of 8.4 C. At similar temperatures the majority of hatched larvae are normal between salinities of about 20 and 35‰ (Fig. 5). Therefore, on the basis of available hydrographic information salinity alone would not appear to limit development of eggs over the geographic range of the English sole.

Mean sea surface temperatures in February for the coastal regions of the area considered range from near 0 C in the eastern Aleutian Islands of Alaska to 14–15 C in the region of the United States–Mexico border (Anon., 1962; Hollister, MS, 1964). Water temperatures in the month of February in Sebastian

Vizcaino Bay, Mexico (10-m depths, 1950–59) are estimated to be 15–17 C (Anon., 1963), surface temperatures being probably in the range of 17–18 C (Sverdrup et al., 1946). Over the range of the commercial fishery for English sole (northern Hecate Strait to lower central California) mean sea surface temperatures for February range from about 6.4 C at Triple Island in Hecate Strait (Hollister, MS, 1964) to 13.3 C at Santa Barbara, California (Anon., 1962).

On the basis of the experimental evidence, temperatures above and below those associated with the 90% isopleth for viable hatch (6.5–10 C) will have a marked effect on egg survival at salinities between 20 and 35‰. Limiting temperatures, insofar as commercial quantities of English sole are concerned, might be considered those at which 50% or less of viable larvae were produced in this study. Such temperatures were calculated as 4.5 C or lower and 12.5 C or higher at salinities approximating 26‰ (see Fig. 7). The hydrographic records of mean sea surface temperature for February indicate that temperatures of 4.5–12.5 C extend approximately from Sitka, Alaska to Point Conception, north of Santa Barbara, California (see Fig. 8).

One further point of interest emerges from comparison of the above hydrographic records and the experimental results of hatching success. The salinity–temperature interaction shown for total hatch, and suggested for viable hatch, corresponds with the trend of coupled increases in salinity and temperature from the northern to the southern part of the coastal region considered. That is, the low/low–high/high interaction effect of salinity and temperature on hatching success is associated with north–south salinity–temperature trends in the natural environment. This relationship would be of some advantage to developing eggs near the northern and southern extremities of the geographic range where temperature levels could be critical to survival of eggs.

Figure 9 compares three sets of data: (A) the laboratory results on viable hatch (from the temperature-viable hatch relationship calculated on the 25.9‰ S transect, Fig. 7); (B) the average sea surface temperature during the month of February around the northeastern Pacific from western Alaska to the border between California and Mexico (Anon., 1962; Hollister, MS, 1964); and (C) the average annual landings of English sole grouped by roughly 300-mile intervals. The projected area of hypothetical abundance compares favourably with the areas in which most of the English sole catch is obtained (see Fig. 8).

Lack of a North American trawl fishery in several Alaska areas restricts full consideration of the relationship which may exist at the northern end of the commercial range. Results of several exhaustive surveys of these regions (Anon., 1964) have revealed the presence of English sole in varying quantities as far west as waters south of Unimak Pass in western Alaska (Fig. 8). However, there was nothing to support the view that abundance is anywhere near equal to that in areas from British Columbia southward. In the southern part of the range it would appear that development of the pelagic eggs would be

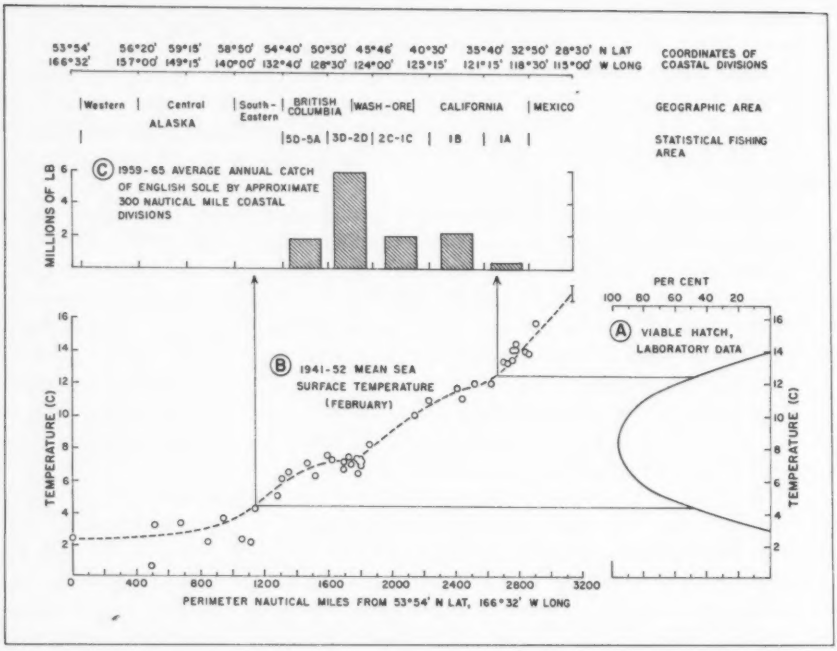
53°54' 56°20' 59°15' 58°50' 54°40' 50°30' 45°46' 40°30' 35°40' 32°50' 28°30' N LAT
166°32' 157°00' 149°15' 140°00' 132°40' 128°30' 124°00' 125°15' 121°15' 118°30' 115°00' W LONG
COORDINATES OF COASTAL DIVISIONS
Western | Central ALASKA | South-Eastern | BRITISH COLUMBIA | WASH-ORE | CALIFORNIA | MEXICO
GEOGRAPHIC AREA
5D-5A | 3D-2D | 2C-1C | 1B | 1A |
STATISTICAL FISHING AREA
C 1959-65 AVERAGE ANNUAL CATCH OF ENGLISH SOLE BY APPROXIMATE 300 NAUTICAL MILE COASTAL DIVISIONS
MILLIONS OF LB
B 1941-52 MEAN SEA SURFACE TEMPERATURE (FEBRUARY)
A VIABLE HATCH, LABORATORY DATA
PER CENT
100 80 60 40 20
TEMPERATURE (C)
0 400 800 1200 1600 2000 2400 2800 3200
PERIMETER NAUTICAL MILES FROM 53°54' N LAT, 166°32' W LONG

FIG. 9. Relationship between (A) laboratory data relating temperature and viable hatch (%); (B) mean sea surface temperatures for the month of February; and (C) catch of English sole by approximate 300 nautical mile divisions of the coastline. Surface temperatures and catch intervals are related to a scale of perimeter nautical miles from Dutch Harbor, Alaska (53°54′ N lat, 166°32′ W long) to Sebastian Vizcaino Bay, Mexico (28°30′N lat, 115°00′W long). Relative geographic and statistical fishing areas are indicated. The arrows relate the region of expected English sole abundance (C) to the range of surface temperatures (B) within which 50% or greater hatch of viable larvae would be expected from laboratory results (A).

completely inhibited by temperature from the vicinity of the United States–Mexico border southward (Fig. 9). Temperature tolerance of larval and adult English sole is unknown. General temperature relationships in other species, however, suggest that larval or adult sole would be tolerant to a wider range of temperatures than the eggs of the species. On the basis of the present experimental evidence it is assumed that the occurrence of limited numbers of English sole in Mexican waters south to Sebastian Vizcaino Bay may result either from larval drift or from migration of older individuals from more northerly waters.

A more detailed examination of records of the North American fishery (California to British Columbia) reveals marked irregularities both in catch and estimated abundance from area to area (Fig. 8). These irregularities are not in accord with the general north–south temperature gradient along the North American coast, suggesting that one or more of the assumptions listed earlier may not be valid. Presumably factors other than the direct

effect of temperature on the viability of eggs also are of importance. For example, the relative scarcity of English sole in the regions of British Columbia designated by statistical fishing areas 3C, 3D, 5A, 5B, and 5C (Fig. 8) may be a consequence of the lack of suitable rearing areas (shallow, sandy bottom) for the initial demersal stage after metamorphosis.

In a study of the English sole population of northern Hecate Strait (area 5D), an area where catch and abundance are above average in relation to other areas along the coast, Ketchen (1956) noted the existence of an extensive rearing area for the early demersal stages. He also noted an apparent inverse relationship between year-class strength and water temperature during the pelagic stage. He concluded that low temperatures favoured survival by prolonging the pelagic stage, thus permitting more young to be transported to the rearing area in the northern part of the Strait.

SUMMARY

1. Fertilized eggs of the English sole (*Parophrys vetulus*) were held to completion of hatching in various combinations of constant salinity (10–40‰ S) and constant temperature (4–12 C).

2. Hatching occurred in all salinities and temperatures except 2 C. Survival to hatching was highest in the midranges of salinities and temperatures and lowest at a temperature of 4 C.

3. Development time to 50% hatching ranged from approximately 3.5 days at 12 C (25‰ S) to 11.8 days at 4 C (25‰ S).

4. Rate of development (the reciprocal of time in days to 50% hatch × 100) was directly related to temperature and the relationship showed slight curvature for temperatures of 4–12 C.

5. Between temperatures of 6 and 12 C development time to 50% hatch appeared to be delayed by changes in salinity above and below 25‰. At 4 C, hatching appeared to be accelerated by changes in salinity above and below 25‰.

6. The greatest mean larval length, 2.92 mm, was obtained in a test at 25‰ S, 8 C. Calculation of a response surface from all data suggested that larvae would reach a maximum size at or in the vicinity of 28.1‰ S and 7.9 C.

7. Total hatch also was greatest in the 25‰ S, 8 C test. Calculation of a response surface suggested that maximum total hatch of about 97% would be obtained at or in the vicinity of 25.6‰ S and 9.0 C.

8. The effect of a change of 1 C on total hatch was calculated to be equivalent to that of a change of 4‰ S. In this respect eggs may be considered euryhaline with respect to salinity and less tolerant to changes in temperature. There was a marked interaction between salinity and temperature.

9. The calculated temperatures at which maximum total hatch could be expected with respect to salinity within the range of 15–35‰ are:

S(‰)	15	20	25	30	35
T(C)	8.6	8.8	9.0	9.2	9.5

10. Viable hatch (of normal larvae) also was greatest in the test at 25‰ S, 8 C and the maximum was calculated to be about 96% at the centre of the response surface, at or in the vicinity of 25.9‰ S, 8.4 C. Changes of 1 C were equivalent to changes of 3.5‰ S in their effect on viable hatch and there was no significant interaction between salinity and temperature.

11. English sole eggs sank just prior to hatching in 30‰ S. Further trials showed an increase in specific gravity of the egg from time of fertilization until hatching. Salinity of neutral buoyancy increased from 27.8 to 30.0‰ during this period.

12. Oxygen consumption of English sole eggs was estimated to be about 0.560 mg/g of embryo per hr. Perfusion velocities in the present study were adequate to ensure independence of response over levels of oxygen supply.

13. Salinities and temperatures encountered by the English sole egg in the natural environment were estimated to range from approximately 20 to 34‰ S and 2.3 to 13.8 C. On the basis of experimental evidence presented, the calculated ranges of salinity and temperature associated with 90% viable hatch are 20–32‰ S and 6.5–10 C. Although salinity may modify effects of temperature on early development it appears to have little direct influence on egg survival.

14. Range and abundance of English sole appear to be influenced by temperature during the period of egg development. From hydrographic records temperatures of 6.4 and 13.3 C are associated with the northern and southern boundaries of the commercial range. By comparison, from the present experimental data, temperatures associated with 50% or greater viable hatch are calculated to range from 4.5 to 12.5 C. Temperature found at the limits of the geographic range are estimated at 2.3 and 18 C; those associated with experimental estimates of zero viable hatch are 3 and 14 C. It is suggested that the extremes of the geographic range may be populated through larval drift or migration of older individuals from the more central part of the range. Thus, temperature may act as a limiting factor to egg production at the northern and southern boundaries of commercial abundance and as a lethal factor near the extremes of the geographic range.

15. Irregularities in catch and abundance of English sole over the commercial range indicate that other factors besides temperature may influence egg or larval survival. The existing literature points toward two such influences: the presence and extent of areas suitable for continued larval development, and water transport phenomena which may move drifting eggs toward or beyond such rearing areas.

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Absorption Coefficients of Chlorophyll Derivatives[1]

By Seward R. Brown

Department of Biology
Queen's University, Kingston, Ont.

ABSTRACT

Methods are described for the isolation of chlorophylls *a* and *b*, and for the preparation of 12 chlorophyll derivatives, including crystalline pheophorbide *a*, from these starting compounds. Laboratory cultures of *Chlorella* were fed $C^{14}O_2$. After 9 days growth the cells were heat-killed, their pigments extracted, and chlorophylls *a* and *b* isolated chromatographically. The following C^{14}-labelled compounds were prepared: chlorophyll *a*, allomerized chlorophyll *a*, chlorophyllide *a*, pheophytin *a*, allomerized pheophytin *a*, pheophorbide *a*, methyl pheophorbide *a*, chlorophyll *b*, chlorophyllide *b*, methyl chlorophyllide *b*, allomerized chlorophyllide *b*, allomerized methyl chlorophyllide *b*, pheophorbide *b*, and allomerized pheophorbide *b*. Assuming uniform distribution of carbon-14 label in the parent chlorophyll molecules, specific and molar absorption coefficients were calculated from the specific radioactivities of these derivatives.

INTRODUCTION

It is now clear that the chlorophylls of phytoplankton undergo diagenesis during sedimentation of cells from the water column, and that diagenesis continues as cellular materials accumulate to form the organic fraction of lake sediments (Brown MS, 1962; Patterson and Parsons, 1963; Brown et al., 1964; Yentsch, 1965a). Comprehensive studies of the breakdown products or their distribution have not yet been reported, but there is general recognition of the fact that extracts of phytoplankton and sediments may contain mixtures of chlorophylls and their derivatives. Arbitrary units have been employed in measurement of such mixtures (Vallentyne, 1957, 1960; Orr et al., 1958; Gorham, 1959, 1960; Swain et al., 1964), usually without identification or separation of the individual components. A few studies (e.g. Corcoran, MS, 1957; Patterson and Parsons, 1963) report specific measurements of pheophytins, chlorophyllides, and pheophorbides after chromatographic separation of these pigments. Other chlorophyll derivatives, including oxidized forms, have been isolated both from phytoplankton and from sediments. The composition and concentration of the derivatives vary with source of extracted materials, indicating potential interest to studies of primary productivity, algal senescence, and the sedimentary processes involved in eutrophication.

Where measurement of primary productivity is based on the pigment content of phytoplankton, it appears to be significant to distinguish chlorophylls, often in very low concentration, from their total non-photosynthetic derivatives. For this purpose, fluorometric measurement (Yentsch and Menzel, 1963; Yentsch, 1965b; Holm-Hansen et al., 1965), because of its sensitivity, is

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markedly superior to spectrophotometry. In contrast, if the object of study is pigment diagenesis, whether in senescent cells, detritus, or sediments, much higher concentrations of pigments are required, and recognition of individual pigment components is of primary importance. For identification and measurements of these components spectrophotometry is clearly the method of choice but is hampered by the paucity of physical data necessary for such measurement.

There are minor difficulties in separation of bulk quantities of chlorophylls and their derivatives by column chromatography but small quantities can be isolated readily by paper and thin-layer techniques (Holden, 1965). Identification of the compounds is complicated by the fact that within the *a* and *b* series the absorption spectra (in the visible range) of chlorophyll, chlorophyllide, and chlorophyllide esters are indistinguishable, as are those of the pheophytin, pheophorbide, and pheophorbide esters. Therefore, identification cannot be established from absorption spectra alone, but requires correlative chromatographic or chemical data. The major obstacle in the study of chlorophyll diagenesis is encountered in quantitative measurement of the derivatives. Available quantities are much too small to permit gravimetric measurements, and a knowledge of specific absorption coefficients is necessary for photometric determinations. The present status of that knowledge is unsatisfactory. For some pigment solutions absorption coefficients are available only for a given solvent; for other compounds they have not been determined. Stern and Wenderlein (1935a, b, 1936) reported absorption coefficients for pheophytin *a* and methyl pheophorbides *a* and *b*, in dioxane; and they were given by Mackinney (1940) for chlorophylls *a* and *b*, in acetone. Coefficients in diethyl ether solution were reported by Zscheile and Comar (1941) and Smith and Benitez (1955) for chlorophylls *a* and *b* and pheophytins *a* and *b*, and by Holt and Jacobs (1954) for the ethyl chlorophyllides and pheophorbides. For other compounds, notably the oxidised or allomerised derivatives which have not been crystallised, no useful physical data exist in the literature.

The present study was undertaken in an effort to provide further data on extinction coefficients of chlorophylls and their derivatives, particularly the allomerised forms, as well as to furnish authentic reference compounds for comparison with pigments extracted from algae and lake sediments. The approach used was to extract C^{14}-labelled chlorophylls *a* and *b* from algal cells, and from these starting compounds to prepare and isolate as many as possible of the more common derivatives. For each pigment optical densities, at the appropriate absorption maxima, were plotted against radioactivity. Crystalline pheophorbide *a* was then prepared and its extinction coefficient determined conventionally by plotting optical density against concentration. The relation between optical density and radioactivity already having been established, it was then possible to relate radioactivity to weight of carbon in the pheophorbide, and from this determination of specific activity to calculate extinction coefficients for the other derivatives which had been prepared. These calculations are based on the assumptions that molecular weights of

the derivatives are correct as reported and that there was uniform distribution of the C^{14}-label in the parent chlorophylls.

MATERIALS AND METHODS

Preparation of C^{14}-labelled Chlorophylls

Cultures of *Chlorella* were grown in Myers' solution (Myers et al., 1951) under constant illumination in glass-stoppered Fernbach flasks to which $C^{14}O_2$ was supplied. After 9 days growth the algal cells were heat-killed, centrifuged, and extracted with 90% acetone in saturated aqueous $MgCO_3$. The cellular material was removed by filtration, an equal volume of diethyl ether added to the acetone extract, and phase separation of the solvents achieved by addition of distilled water containing 10% NaCl. The ethereal layer containing the chloroplast pigments was washed three times with distilled water saturated with $MgCO_3$, then reduced to about 10% of its original volume before drying over anhydrous Na_2SO_4. When storage was necessary, at any stage in the preparation, the pigment solutions were kept in the dark at 0 C in diethyl ether.

Column chromatography was used for the initial separation of pigments, following the method described by Jacobs et al. (1954). The chromatographic separations and all subsequent operations were carried out at room temperature in dim light. All solvents used were reagent-grade, but the further preparative precautions recommended by Zscheile (1934) were observed. A total of eight colored bands containing chlorophylls, chlorophyll derivatives, and carotenoid separated on the columns. Development was allowed to proceed until the chlorophyll *a* fraction had passed into the receiving flask, at which point the columns were dried briefly with nitrogen, then extruded, and the chlorophyll *b* band cut out. The sugar slice containing the chlorophyll *b* band was extracted with acetone, then quickly transferred to diethyl ether. Spectroscopic examination of both the chlorophyll *a* and *b* fractions showed contamination with other pigments, indicating the necessity for further chromatography. Previous experience had shown that for further resolution of small quantities of pigments a higher degree of uniformity and purity of preparation could be achieved on paper than by use of chromatographic columns.

Paper chromatography of chloroplast pigments has been reviewed by Sestak (1958), who cites evidence invalidating the use of R_f values as a means of identifying zones of pure pigment. The order in which pigments appear on paper chromatograms is modified by the type of developing solvent; and the R_f of any component compound of the sample, in a given solvent system, is affected by its concentration and the kind and concentration of other pigments in the sample. In the present study, two solvent systems were found to be especially useful. The first consisted of 3–35% PPP (6% propanol, 4% pyridine, and 90% petroleum ether (60–75 C)) in petroleum ether (30–60 C). Resolution of chlorophyll *a* fractions was regularly done with 10% PPP, whereas 25–36% PPP was used for chlorophyll *b*. The other system consisted of 1–5% 1,2-di-

chloroethane in petroleum ether (60–75 C), the higher concentrations of dichloroethane being used for resolution of chlorophyll *b*.

Chlorophyll fractions, previously separated on sugar columns, were reduced under vacuum to small volume, then streaked on 23 × 57-cm sheets of Whatman No. 3 MM paper. Separations were made on descending chromatograms, in the dark, at 15 C. Most satisfactory separations were achieved when the paper had been allowed to equilibrate with the atmosphere of the developing solvent for a minimum of 20 min before beginning development. Preliminary tests showed no advantage in flushing the chromatography cabinet with nitrogen, or in the addition of 0.5% dimethylaniline (Strain, 1954) to the developing solvents. Particular attention was paid, however, to preliminary purification of solvents, speed in preparation of chromatograms, and care to avoid drying while preparing pigments for streaking. After resolution of the pigments, each band was cut out of the paper and eluted with anhydrous diethyl ether. Spectroscopic determinations were made in ether solution on a Cary recording spectrophotometer, Model 11S. Radioactivity measurements were made on porcelain planchettes in a Nuclear Measurements Corporation alpha-beta-gamma proportional counter, Model PC-1. Duplicate samples were plated and a minimum of 10,000, most frequently more than 30,000 counts, were recorded.

After chromatography of chlorophyll *a* with the PPP solvent system, only a shoulder was observed at 410 mμ (in ether) instead of the peak reported by Zscheile and Comar (1941) and Smith and Benitez (1955). The pigment was then rechromatographed using 2% dichloroethane in petroleum ether. Again only a shoulder appeared at 410 mμ but its height was reduced by 10% in comparison with that of the previous preparation. There was also a 15% reduction in the radioactivity of this preparation, presumably through removal of colorless contaminants. Repeated chromatography with PPP, dichloroethane-petroleum ether, and other solvent systems produced no further change in the absorption spectrum or in specific radioactivity. Chlorophyll *a* carrier was then prepared in exactly the same way as the labelled pigment, and mixed with the latter in 3:1 proportions. The line spectrum obtained from the resultant mixture was identical with that of the C^{14}-labelled preparation.

A constant ratio between the blue and red absorption maxima was obtained when the chlorophyll *b* fraction was chromatographed first in PPP and then in dichloroethane–petroleum ether solvent systems. Further chromatography in 36% PPP resulted in reduction of the height of the accessory peak at 430 mμ, and a reduction of 23% in radioactivity. Repeated chromatography in a variety of solvents showed no further changes in absorption spectrum or in specific activity. Chlorophyll *b* carrier was prepared and a threefold dilution made, as had been done with chlorophyll *a*. Figures 1 and 2 show the relations between radioactivities of the preparations and their absorption maxima, in a series of concentrations.

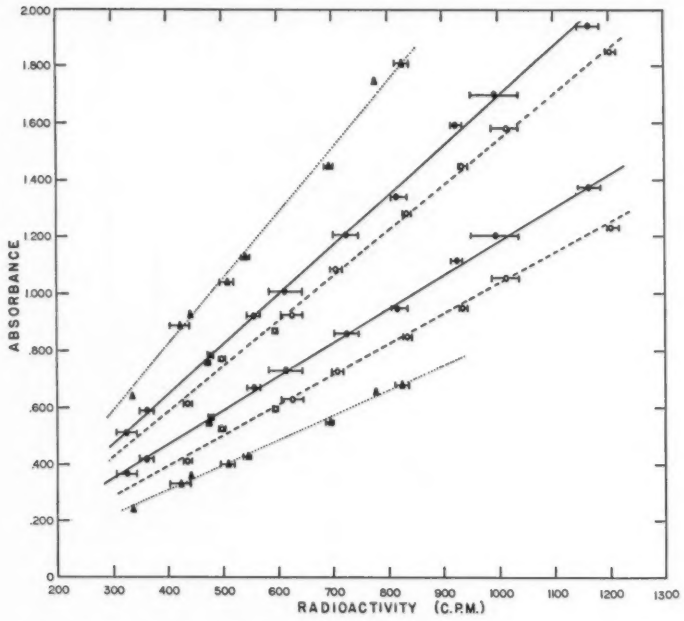

FIG. 1. The relation between radioactivity and absorbance, at the blue (upper line) and red (lower line) absorption maxima, in ether solutions, of: chlorophyll *a* (———); chlorophyllide (– – – –); allomerized pheophytin *a* (.....). In this and the subsequent figures the symbol (—●—) denotes maximum· range of counts.

PREPARATION OF CHLOROPHYLL DERIVATIVES

The chlorophyll *a* and *b* preparations described above were used as starting compounds for production of the following series of derivatives. Techniques were first tested and an authentic set of reference samples collected in a trial run using unlabelled chlorophylls. The general scheme of degradation is shown in Fig. 3.

These derivatives are phorbins, that is, the cyclopentanone Ring V remains intact, whereas in chlorins the ring is opened to form two additional carboxyl groups or esters. Phorbins are the products of three basic reactions or combinations of reactions: (*a*) oxidation at carbon-10 to produce allomerized chlorophylls; (*b*) removal of magnesium, readily from chlorophyll *a* and with slightly more difficulty from chlorophyll *b*, to produce pheophytins; and (*c*) removal or replacement of the phytyl group by the enzyme chlorophyllase, to produce chlorophyllides. Pheophorbides are formed on loss of both magnesium and phytol from the chlorophyll molecule.

PHEOPHYTINS

Pheophytins were prepared by the method given in detail by Smith and Benitez (1955) except that the reaction was carried on only for a period of

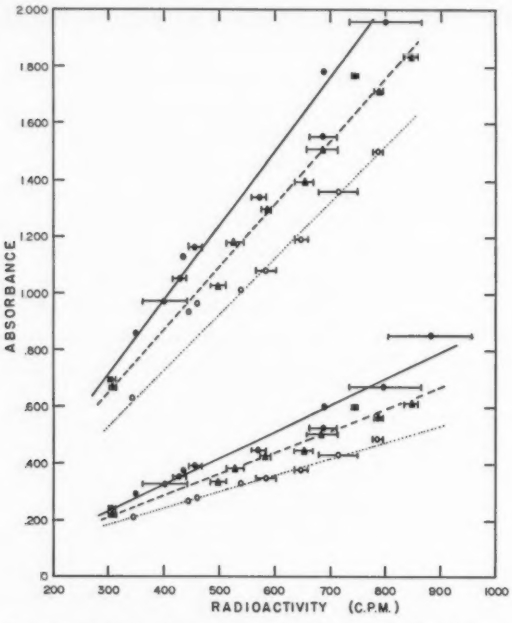

FIG. 2. Relation between radioactivity and absorbance, at the absorption maxima, in ether solutions, of: chlorophyll *b* (———); chlorophyllide *b* (.....); methyl chlorophyllide *b* (– – – –).

6 hr. The pheophytin solutions so obtained were reduced to small volumes, dried over anhydrous Na_2SO_4, and chromatographed on Whatman No. 3 MM paper.

Development of pheophytin *a* with 4% PPP resulted in only one visible band of pigment, although a second band of very low concentration was observed ahead of it when chromatograms were inspected under ultraviolet light. When the major band was rechromatographed there was no further evidence of the second band, indicating the formation of the second compound during the acid reaction and not degradation in the process of chromatography. The spectrum of the pheophytin was in agreement with that of Smith and Benitez (1955). Radioactivities of a series of aliquots were measured and are shown plotted against absorbance at the blue and red maxima in Fig. 4.

Pheophytin *b* was chromatographed in 10% and 15% PPP. On first chromatography three narrow bands of low concentration were observed in addition to the major band. All four bands gave absorption spectra with maxima at the same wavelength, but with slight variations in ratios of absorbance at the blue and red peaks. Further chromatography on Whatman No. 1 paper eliminated all but the major band, which gave an absorption spectrum

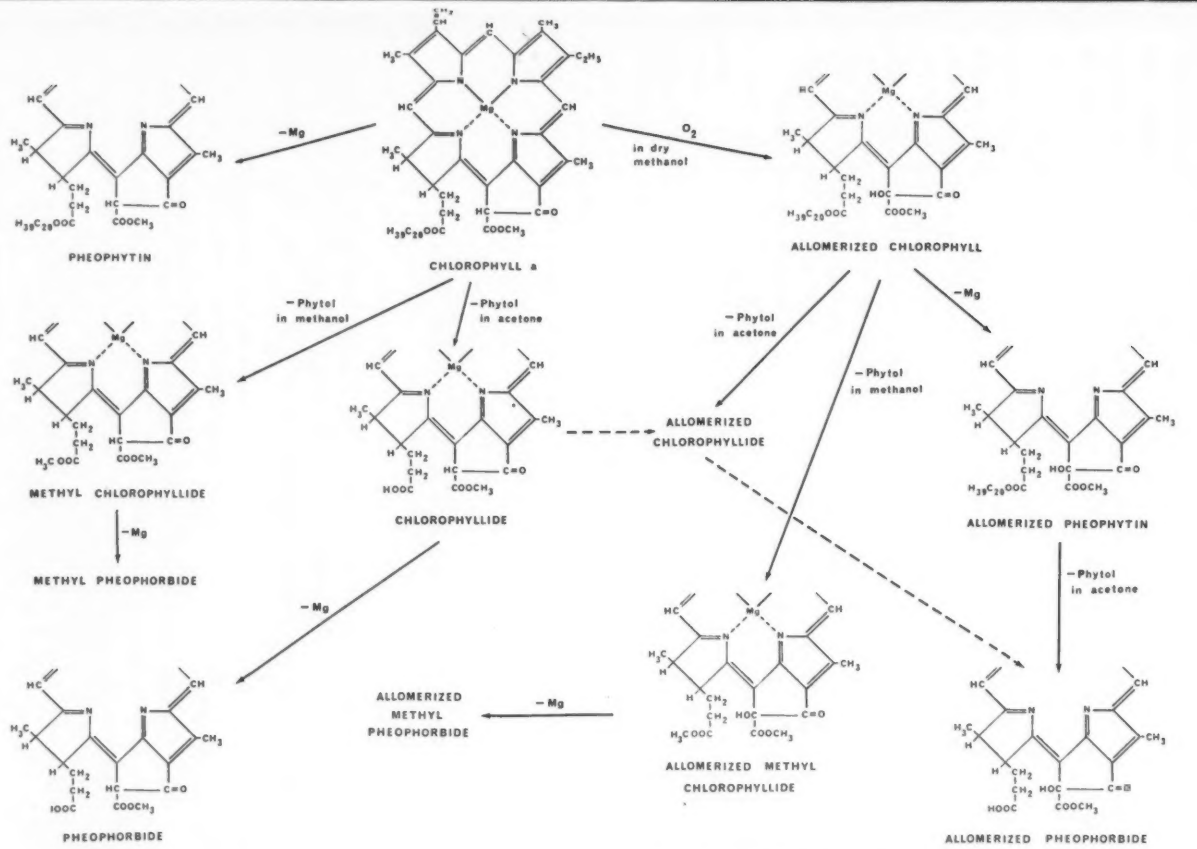

FIG. 3. Scheme of degradation followed in preparation of chlorophyll derivatives.

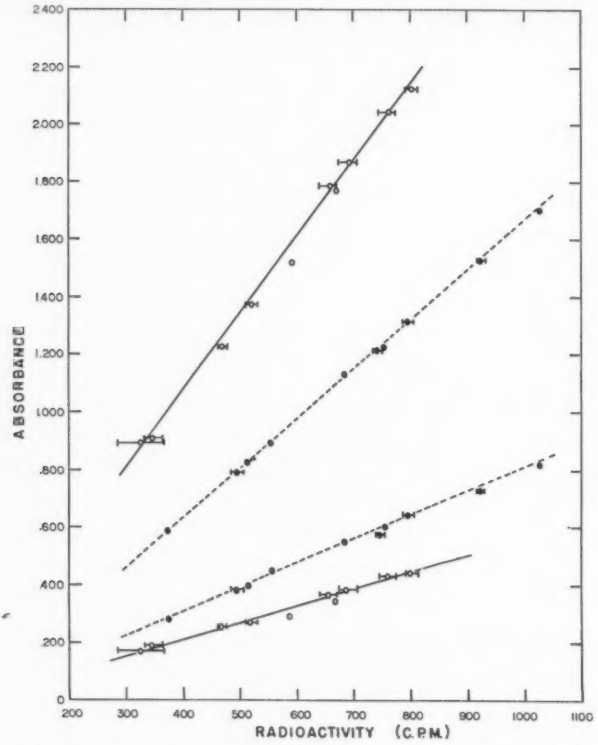

FIG. 4. Relation between radioactivity and absorbance, at the absorption maxima, in ether solutions, of pheophorbide *b* (———); pheophytin *a* (– – – –).

in exact agreement with that of Smith and Benitez (1955). Accidental loss of the pigment made it impossible to obtain measurements of radioactivity over the range of concentrations reported in this paper for other chlorophyll derivatives.

CHLOROPHYLLIDES

Chlorophyllase specifically attacks the phytyl group of the chlorophyll molecule. Depending upon the solvent in which the reaction is carried out, phytol is replaced by methyl, ethyl, or longer chain alcohols to produce esters. In acetone the acid chlorophyllide is formed. *Fucus* gathered near New Haven, Connecticut, in October and May showed high chlorophyllase activity, and was used in all chlorophyllide preparations described below. Thalli were washed, reproductive tips cut off, and the vegetative structures homogenised in a Waring Blendor with methanol. The pulp was separated from the liquid and

repreatedly extracted alternately with 95% methanol and 95% acetone at room temperature, until extracts showed no trace of pigment on spectroscopic examination. The bleached pulp was moistened with 90% methanol or 90% acetone and stored at 0 C. There was no apparent loss of enzymic activity in this material after 3 months storage, whereas dried pulp was inactivated in less time.

In preparation of methyl chlorophyllides, aliquots of the chlorophylls were reduced to small volumes, taken up in 25 ml of 95% methanol, and allowed to stand in contact with 10 g of *Fucus* pulp for 3 hr at room temperature. The mixture was filtered under suction and the pulp washed three times with 25-ml portions of 95% methanol. The pigments were transferred to diethyl ether and washed free of alcohol with distilled water which had stood over $MgCO_3$. The ether solution was dried over Na_2SO_4 and reduced to small volume prior to chromatography. Acid chlorophyllides were prepared by the same technique except that 95% acetone was used instead of methanol.

No particular difficulty was experienced in chromatography of either the methyl or acid chlorophyllide *a*. The acid chlorophyllide had a lower R_f value than the methyl ester, and any residual chlorophyll in either preparation moved easily ahead of the derivative. In both preparations minor bands were detected by their fluoresence under ultraviolet light after chromatography on Whatman 3 MM paper with 36% PPP. The major bands were eluted and chromatographed in 10% dichloroethane–petroleum ether. Only a trace of pigment was observed in a minor band which separated clearly ahead of chlorophyllide *a*. A single band was formed by the acid chlorophyllide. Data for absorbance and radioactivities of chlorophyllide *a* preparations are given in Fig. 1.

The *b* chlorophyllides moved with lower R_f values than the corresponding *a* derivatives, but equally satisfactory separations were achived with the same chromatographic technique. Two minor bands separated ahead of methyl chlorophyllide *b* on chromatography with 36% PPP. Only one appeared on the acid chlorophyllide chromatograms. No further separation was achieved by repeated chromatography with an extensive series of solvents which included: PPP; acetone (4%), dichloroethane (10%), or benzene (15%) in petroleum ether; and *n*-decane: 1-propanol, 6:1 (v/v), as used by Green (1958, and personal communication). Therefore, it appears that chlorophyll *b* is more stable under the preparative conditions employed than is chlorophyll *a*. Data on absorbance and radioactivities are given in Fig. 2.

Pheophorbides

Pheophorbides were prepared from the appropriate chlorophyllides in precisely the same manner as were the pheophytins from their respective chlorophylls. Methyl pheophorbides were chromatographed in 25% PPP, and the acid pheophorbides in 25–40% PPP. Very small quantities of pigments, similar in spectral characteristics to the pheophorbides, separated slightly ahead of the major bands. Complete resolution of these bands was accomplished

on further chromatography in PPP — diethyl ether — petroleum ether, 15:25:60 (v/v). See Fig. 4, 5, and 6 for data on absorbance and radioactivities.

Allomerised Chlorophylls and their Derivatives

Allomerised derivatives are formed when chlorophylls, in anhydrous alcohols, are oxidised by air or oxygen (Conant et al., 1931; Fischer and Reidmair, 1933). The reaction is favored by the presence of magnesium in the molecule, consequently chlorophylls are better starting compounds than pheophytins for the preparation of oxidised derivatives.

Chlorophylls dissolved in anhydrous methanol were either allowed to stand in loosely stoppered flasks or had oxygen slowly bubbled through them, in the dark, at room temperature, for 6–12 hrs. The pigments were then transferred to diethyl ether, washed free of alcohol, and dried over Na_2SO_4. Spectroscopic examination of the preparations showed that quantitative conversion to the allomerised form was rarely achieved, so that a resolution of the mixture was required.

Preparations from unlabelled chlorophylls were first chromatographed on columns of powdered sugar, but because of much smaller available quantities of the labelled compounds these were separated on paper. Chlorophyll *a* was slightly less strongly adsorbed on sugar than the bluish-green allomerised product, and separated fairly satisfactorily on development with 0.5–2% propanol in petroleum ether. Further chromatography on Whatman No. 1 paper in 10% PPP resulted in the complete fractionation of the mixture. For paper chromatography alone, sequential development with 2% acetone in petroleum ether, 10% PPP, 4% acetone, and 20% PPP was used. Absorbance and radioactivity data for allomerised chlorophyll *a* are given in Fig. 5.

The same technique was used in the separation of allomerised chlorophyll *b* from the chlorophyll. Sugar columns were developed with 4–10% propanol in petroleum ether, and the eluate rechromatographed on paper with 20% PPP. When the mixture was separated entirely on paper, successive chromatograms were developed with 5% acetone in petroleum ether and 36% PPP.

The preparative and chromatographic techniques employed for allomerised chlorophyll and its derivatives were essentially the same as those used in dealing with nonoxidised chlorophyll series. Some noteworthy differences in the reactions were observed, however. An instantaneous color change from blue-green to olive-green was observed when ether solutions of allomerised chlorophyll *a* were acidified to produce the pheophytin, and quantitative conversion to the pheophytin was accomplished in 30 min. Allomerised chlorophyll *b* and its chlorophyllides were more strongly adsorbed than the corresponding non-oxidised compounds, and much greater difficulty was experienced in their chromatographic separation. There was poor recovery of the chlorophyllides after enzymic degradation of the allomerised chlorophyll, which was particularly significant when only small amounts of the starting compound were available. In contrast, when chlorophyllide was used as the parent compound and allowed to stand in anhydrous alcohol in contact with air or

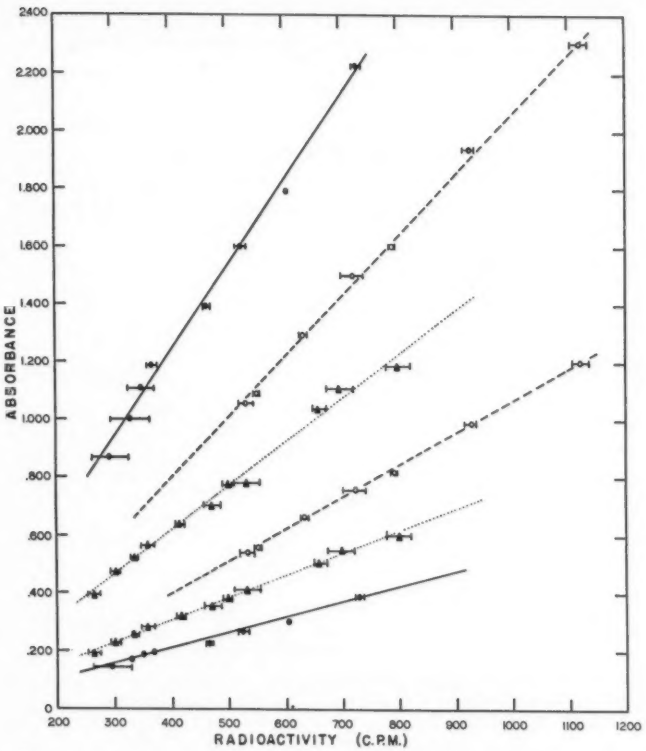

FIG. 5. Relation between radioactivity and absorbance, at the absorption maxima, in ether solutions, of: allomerized chlorophyll *a* (– – –); allomerized pheophorbide *b* (———); pheophorbide *a* (.....).

oxygen, the allomerised chlorophyllide was produced in high yield but the reaction was rather slow. The latter procedure has the disadvantage that the resultant mixture of chlorophyllide and allomerised chlorophyllide is more difficult to resolve chromatographically than is the mixture of allomerised chlorophyll and allomerised chlorophyllide produced by the first reaction. The separation was made, however, by repeated chromatography with 5% methanol — 7.5% acetone — 87.5% petroleum ether. The data given in Fig. 6 were obtained from *b* chlorophyllides prepared in this manner.

PREPARATION OF CRYSTALLINE PHEOPHORBIDE *a*

An algal extract of chlorophyll *a* was separated on sugar columns, eluted, and converted to the acid chlorophyllide by the method described above for the preparation of chlorophyllide *a*. Pheophorbide *a* was then prepared by acidification of the chlorophyllide solution, transferred to ether, and washed

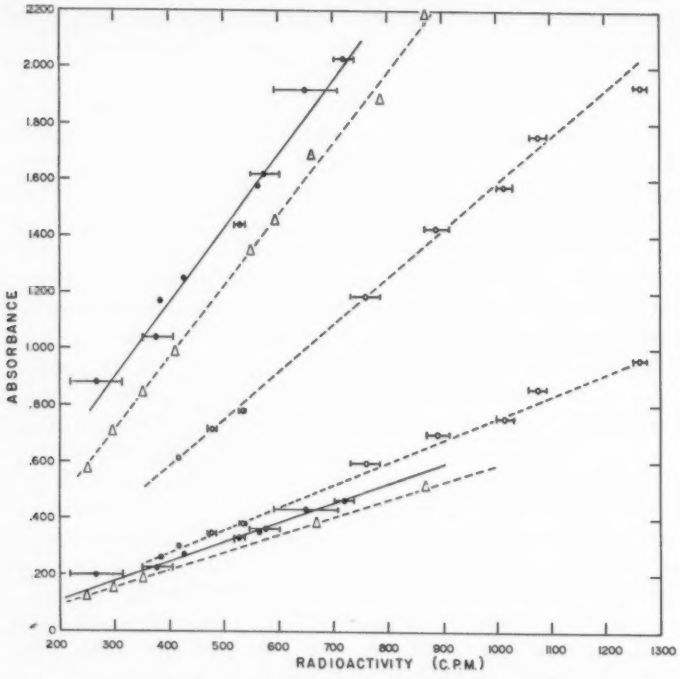

FIG. 6. Relation between radioactivity and absorbance, at the absorption maxima, in ether solutions, of: allomerized methyl chlorophyllide *b* (———); methyl pheophorbide *a* (– – – ○ – – – ○ – – –); allomerized chlorophyllide *b* (– – – △ – – – △ – – –).

with water. Extraction with 15% HCl, which is specific for pheophorbide *a*, produced a violet-colored hypophasic fraction, leaving a brownish-green ether phase. After phase separation, the pheophorbide was transferred to fresh ether solution by addition of distilled water, washed, and dried over Na_2SO_4. The pheophorbide was precipitated by slowly reducing the ether solution, and came down as exceedingly fine microcrystals. Aliquots dissolved in ether and chromatographed with 25% PPP and other solvent systems produced a single band of pigment.

Absorbance measurements were made on four weighed aliquots of crystalline pheophorbide *a* in known volumes of diethyl ether. Dilutions of the solutions were made to extend the range of concentrations. All weighings were made on a Mettler microgram-atic balance, and dilutions made with retested pipets.

Figure 7 shows the absorbance at 667 mμ (the red absorption maximum) plotted against weight of carbon in a series of pheophorbide *a* solutions. On the same graph absorbance is plotted against radioactivity in a series of dilutions of the C^{14}-labelled preparation of the pheophorbide. The chi-square test

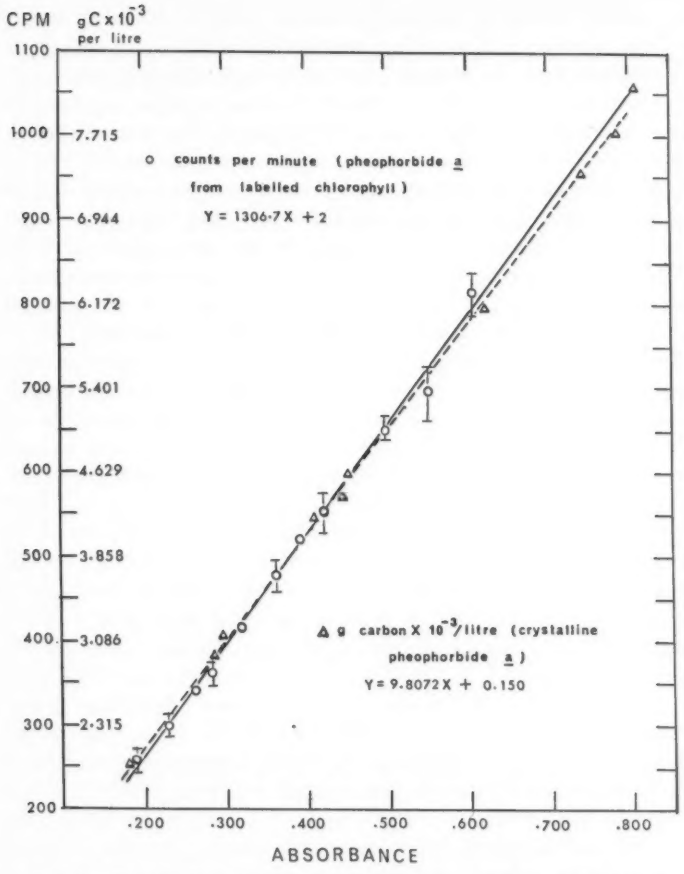

FIG. 7. Relation between radioactivity and weight of pheophorbide *a*.

shows no significant difference ($P = 0.01$) between the data from which the two regression lines were calculated. Thus the relationship is established between weight (grams carbon per litre of solution) and radioactivity (counts per minute) for pheophorbide *a*. The calculations of extinction coefficients for the chlorophylls and their derivatives reported in this paper are based on this specific activity.

RESULTS AND DISCUSSION

The pheophorbide and all other labelled pigments described above were prepared from common starting compounds, the C^{14}-labelled chlorophylls. Therefore, if there was no differential incorporation of C-14 atoms as between the phytol and the tetra-pyrrole nucleus of the chlorophyll molecule, the

specific activity of carbon within the series must be constant, and the same relationship between radioactivity and weight exists in these compounds as in the pheophorbide. The algae from which the starting chlorophylls were extracted were grown in $C^{14}O_2$ for 9 days in order to ensure maximum turnover of carbon consistent with high yield of chlorophyll. The assumption is made in the following calculations that uniform labelling was achieved.

Table I lists the chlorophylls and their derivatives which were prepared, together with their absorption coefficients and the data from which these were derived. The molecular weights used in the calculations are those shown in the first column of the table, and exclude the indefinite quantity of water of hydration known to be associated with the molecule of some crystalline for chlorophyll derivatives. Absorbance was chosen arbitrarily at 0.500 and 0.700 the red and blue absorption maxima of the compounds. From the appropriate regression line the radioactivity of the compound was obtained and converted to potentially labelled carbon per litre of solution. Since this value represents a known percentage of the molecular weight, the weight of the compound per litre of solution was calculated and the specific or molar absorption coefficient thus obtained.

Specific absorption coefficients previously reported are shown for comparison in Table II. It will be noted that all strictly comparable data from other sources give somewhat lower coefficients than those obtained in the present study, and that complete agreement does not exist between any two sources. It is probably more remarkable that agreement should be so close than that there should be a lack of it. For the chlorophylls, only the data reported by Trurnit and Colmano (1959) were obtained from crystalline preparations. Smith and Benitez (1955) calculated chlorophyll concentrations from the magnesium content of the pigment, and the chlorophylls used by Zscheile and Comar (1941), although spectroscopically authentic, were not crystalline. The absorption coefficients of the chlorophylls presented in Table I are based on weights of carbon calculated from crystalline pheophorbide; therefore, any error introduced in this calculation is propagated throughout the series.

Holt and Jacobs (1954) obtained their coefficients from crystalline preparations. They found close aggreement between the molar extinction coefficients of ethyl chlorophyllides *a* and *b* and Zscheile and Comar's (1941) values for the chlorophylls, and similar agreement between their values for the pheophorbides and those reported for the pheophytins by Zscheile and Comar. They therefore conclude that the phytyl group does not influence molar absorption coefficients, and support their conclusion on the theoretical basis that the double bond of the phytyl side chain does not enter conjugation with the double bond system of the porphin ring. The data of Stern and Wenderlein (1936) do not support this argument, nor indeed are they in agreement with the data obtained in this study.

The present study does not resolve the theoretical problem. It provides absorption coefficients for a much wider range of the phorbins than was previously available under comparable conditions of preparation and instrumenta-

TABLE I. Absorption coefficients of chlorophylls *a* and *b* and some of their derivatives. Solvent: diethyl ether; absorbance at "red" maximum: 0.500; absorbance at "blue" maximum: 0.700.

Compound mol wt	Maxima (λ ($m\mu$))	Radio-activity (*counts/min*)	Wt of carbon ($g/liter \times 10^3$)	% carbon potentially labelled	Wt of compounds ($g/liter \times 10^3$)	Sp. Abs. Coeff.[a] $\frac{1}{\bar{g}\ cm}$	Molar Abs. Coeff.[b] ($\times 10^3\ cm^2/M$)
Chlorophyll *a*	428.9	435	3.358	73.93	4.542	154.2	135.8
893.48	662	425	3.281		4.436	112.7	100.7
Allomerised chlorophyll *a*	416	360	2.778	71.37	3.892	179.9	166.5
925.48	654	495	3.819		5.351	93.4	86.5
Chlorophyllide *a*	428	473	3.651	68.35	5.342	131.0	80.6
614.97	662	502	3.874		5.668	88.2	54.2
Pheophytin *a*	408	442	3.411	75.82	4.499	155.6	135.6
871.18	668	641	4.940		6.515	76.7	66.8
Allomerised pheophytin *a*	399	350	2.700	73.13	3.692	189.6	171.2
903.18	671	620	4.783		6.540	76.5	69.1
Pheophorbide *a*	408.5	455	3.510	70.92	4.949	141.5	83.9
592.67	667	655	5.054		7.126	70.2	41.6
Methyl pheophorbide *a*	408.5	465	3.590	69.29	5.181	135.1	82.0
606.69	667	685	5.288		7.632	65.5	39.7
Chlorophyll *b*	453	298	2.301	72.79	3.161	221.4	200.9
907.46	642.5	590	4.555		6.258	79.9	72.5
Chlorophyllide *b*	452	385	2.972	66.83	4.447	157.4	99.0
628.96	641	828	6.392		9.566	52.3	32.9
Allomerised chlorophyllide *b*	441	296	2.285	63.59	3.593	194.8	128.8
660.96	629	865	6.678		10.502	47.6	31.5
Methyl chlorophyllide *b*	452	325	2.509	65.38	3.838	182.4	117.3
642.98	641	690	5.327		8.148	61.4	39.5
Allom. methyl chlorophyllide *b*	441	223	1.722	62.27	2.765	253.2	170.9
674.98	629	790	6.099		9.794	51.1	34.5
Pheophorbide *b*	432	264	2.038	69.29	2.942	237.9	151.9
638.65	654	907	7.002		10.105	49.5	31.6
Allomerised pheophorbide *b*	425.5	225	1.737	65.81	2.639	265.3	177.9
670.65	654.5	960	7.411		11.261	44.4	29.8

[a]Specific absorption coefficient.
[b]Molar absorption coefficient.

TABLE II. Specific absorption coefficients (1/g cm) of phorbins in diethyl ether at red absorption maxima.

Compound	Brown	Holt & Jacobs	Smith & Benitez	Stern & Wenderlein	Trurnit & Colmano	Zscheile & Comar	Mackinney
Chlorophyll *a*	112.7	–	100.9	–	74.0	102.1	84.0 (acetone)
Pheophytin *a*	76.7	–	63.7	49.4 (dioxane)	–	59.0	–
Chlorophyllide *a*	88.2	–	–	–	–	–	–
Ethyl chlorophyllide *a*	–	131.5	–	–	–	–	–
Pheophorbide *a*	70.2	–	–	–	–	–	–
Methyl pheophorbide *a*	65.5	–	–	87.0 (dioxane)	–	–	–
Ethyl pheophorbide *a*	–	59.0[a]	–	–	–	–	–
Chlorophyll *b*	79.9	–	62.0	–	57.4	56.8	51.8 (acetone)
Pheophytin *b*	–	–	42.1	–	–	37.0	–
Chlorophyllide *b*	52.3	–	–	–	–	–	–
Methyl chlorophyllide *b*	61.4	–	–	–	–	–	–
Ethyl chlorophyllide *b*	–	76.9	–	–	–	–	–
Pheophorbide *b*	49.5	–	–	–	–	–	–
Methyl pheophorbide *b*	–	–	–	54.6 (dioxane)	–	–	–
Ethyl pheophorbide *b*	–	37.0[a]	–	–	–	–	–

[a]Not assumed hydrated in this calculation.

tion and, in an internally consistent system, extends these data to include five allomerised compounds not previously studied in this aspect.

ACKNOWLEDGMENTS

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The Aquiferous System of *Polinices lewisi* (Gastropoda, Prosobranchiata)[1]

By F. R. Bernard

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

ABSTRACT

Polinices lewisi (Gould) possesses an aquiferous system for the expansion of the podial complex. The haemal system is highly modified and entirely separate from the aquiferous system. Postpodial ostia and haemal sheaths are demonstrated. The morphology of the aquiferous and blood systems is traced.

INTRODUCTION

Speculation has surrounded the mechanics of foot dilation in the Naticidae. Early theories which held that expansion was due to the uptake of water from the surrounding medium were discarded when Carrière (1882) showed that for many species foot dilation and turgescence was accomplished by the influx of large quantities of blood and hypothesized that this was the situation in all molluscs. Schiemenz (1884) described the aquiferous system in the Naticidae and stated that turgescence was the result of water uptake through small marginal slits in the foot. Because such a system is unique to the naticids, Schiemenz' work was largely ignored. Morris (1950) demonstrated that the Australian naticid *Uber strangei* (Reeve) probably takes in water for foot expansion. Ziegelmeier (1958) in his description of the pedal movements of *Polinices josephinus* Risso noted the exudation of water on the contraction of the foot.

Polinices lewisi (Gould), an important clam predator, was used in this study. The investigation was divided into three phases: (i) the proof of water uptake from the surrounding medium; (ii) the morphology of the aquiferous system and associated modifications of the blood circulatory system; (iii) the mechanics of foot expansion and movement.

MATERIALS AND METHODS

Specimen relaxation was accomplished by the use of 1 Naphthyl-N-methylcarbamate, an insecticide marketed under the name "Sevin." A dilution of approximately 20 parts per million in seawater was found to be effective, the snails being allowed to remain 3 or 4 days in the same water.

Corrosion models of the aquiferous and blood circulatory systems were prepared by injection of a monomer base solution mixed with catalyst which solidifies into a polymer; the flesh was then removed with a solution of potassium hydroxide.

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WATER UPTAKE

Of all naticids *P. lewisi* possesses the largest foot, a pale grey oedematous mass up to four times the total shell volume. On irritation the foot contracts, exuding quantities of fluid and can be completely withdrawn into the shell. This exudable water makes up approximately 43% of the total weight of the expanded snail. Table I lists the expanded and contracted weights of twenty individuals.

It has been suggested by some workers that the fluid lost is blood rather than water, due to the rupture of blood sinuses and vessels, and that full withdrawal of the foot is irreversible and causes the death of the individual. Microscopic examination of the precipitate of centrifuged exudate failed to reveal any appreciable numbers of amoebocytes as would be expected if it were blood. In physical respects the characteristics of the exudant are similar to the surrounding medium in which the snail has expanded.

Repeated experiments with various individuals have clearly demonstrated the expansion/contraction cycle to be repeatable and to have no deleterious effect upon the snail. Table II lists the weights of two individual snails subjected to five cycles of contraction and expansion.

MORPHOLOGY

The large spreading foot, metapodial folds covering the shell and the wedge-shaped propodium are adaptations to existence in and ability to progress through mud and gravel. The external morphology of the *Polinices* foot is well-known and need not be redescribed.

Anteriorly the pedal aquiferous sinus is a large chamber provided with a meshwork of muscular cords (Fig. 1A). Posteriorly, it flattens out and is connected to the exterior via 40–80 ostia (Fig. 1A, *o*) situated on the margin of the postpodium. When liquid plastic is injected in the pedal aquiferous sinus, the entire pedal and circumpharyngeal regions are impregnated but no blood vessels or body haemal sinuses are filled (Fig. 1B). The aquiferous system of *P. lewisi* is separate from and unconnected with the blood circulatory system.

The largest aquiferous sinus is situated in the anterior portion of the propodium and consists of eight interconnected lacunae which branch out to fill the entire podial and metapodial complex. A secondary and smaller sinus surrounds the pharynx and proboscis.. (Fig. 1B, *cps*). Posteriorly the eight lacunae fuse and form a broad thin chamber extending to the edge of the postpodium and ostial margin.

Located on the edge of the postpodium are lateral slits approximately 40 μ in width, the marginal ostia. The edge of the postpodial sinus is supplied with many vertical and lateral muscle bundles which are capable of constriction, closing the ostia in the manner of a sphincter (Fig. 2A, B).

On contraction the propodium is rapidly detumesced and withdrawn into the shell, the volume of water being transferred to the postpodium which then contracts, ejecting water from the ostia with considerable force.

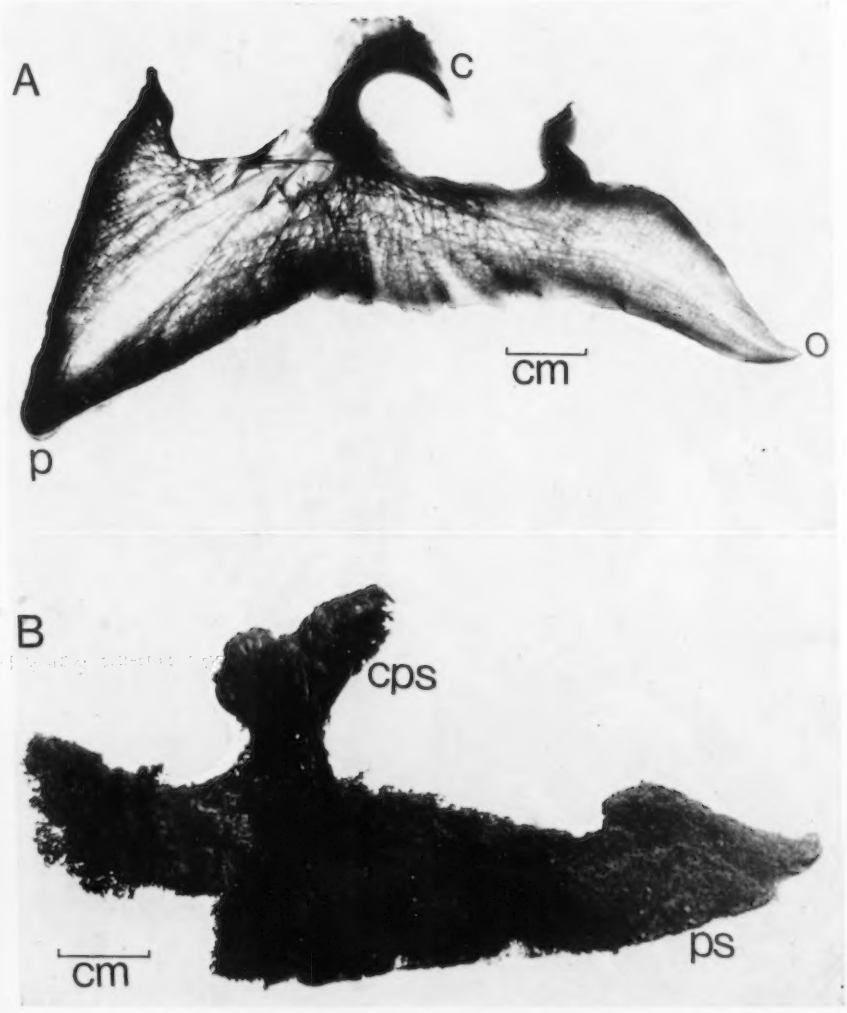

FIG. 1. Aquiferous system of *Polinices lewisi*: A, sagittal section of entire podium; B, corrosion model, made by injection of liquid plastic into aquiferous system.

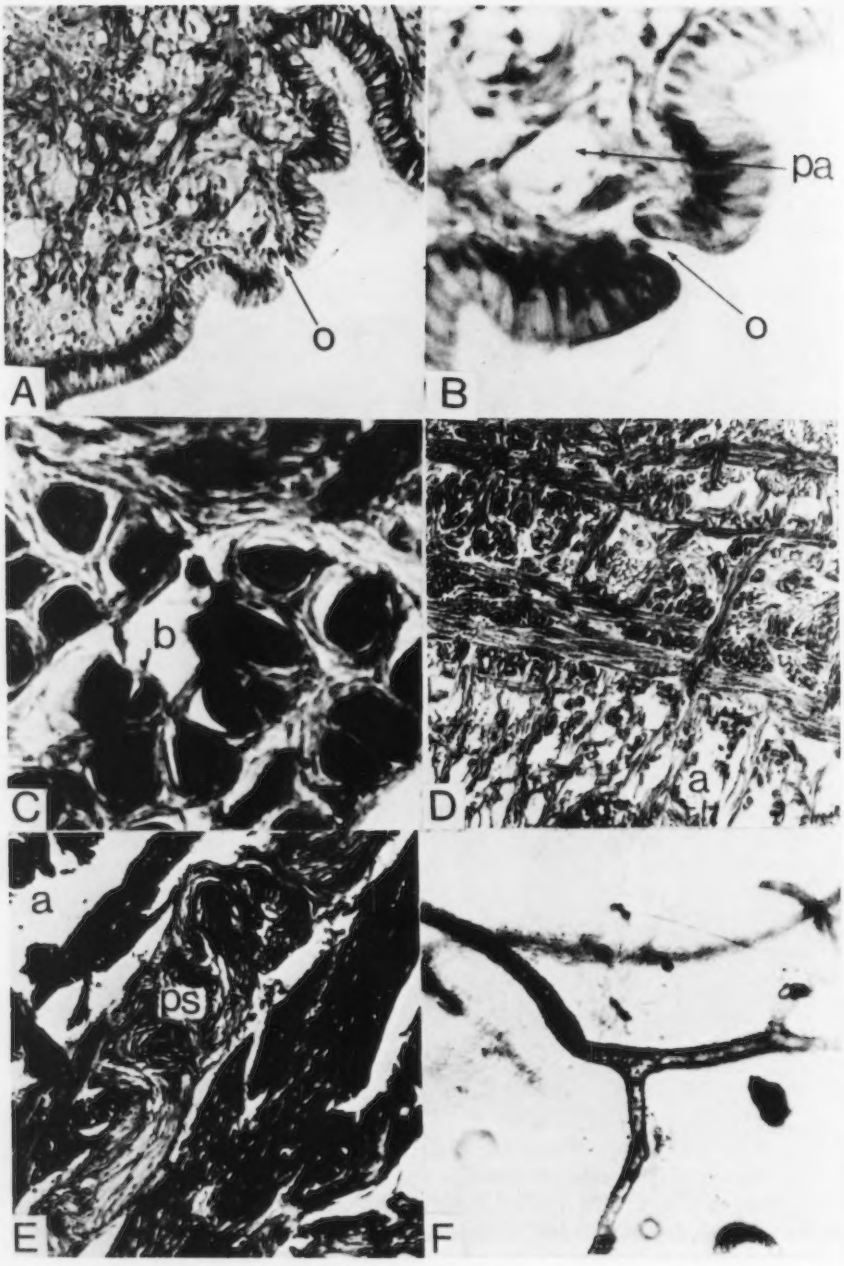

FIG. 2. Aquiferous and haemal systems of *Polinices lewisi*: A, sagittal section of edge of postpodium, showing ostium. Harris's haematoxylin-eosin ×82; B, section of ostium, Harris's haematoxylin-eosin ×182; C, transverse section of relaxed muscle bundle showing haemal sheaths and blood space (*b*). Masson's tri-chrome ×391; D, section of foot showing muscle bundles and aquiferous sinus (*a*). Masson's tri-chrome ×82; E, section of pedal blood sinus, Heidenhain's haematoxylin-eosin ×182; F, plastic model of fine arteries from propodium, tissues cleared in potassium hydroxide ×46.

TABLE I. Expanded and contracted weights (in grams) of 20 *Polinices lewisi*.

	Expanded	Contracted	Difference
1	279	180	99
2	147	69	78
3	210	120	90
4	301	160	141
5	225	120	105
6	310	200	110
7	340	167	173
8	640	322	318
9	201	104	97
10	453	231	222
11	150	93	57
12	343	185	158
13	103	81	22
14	290	175	115
15	410	230	180
16	91	67	24
17	80	53	27
18	120	95	25
19	253	147	106
20	298	186	112
$\bar{x}$	262	149	113

TABLE II. Expanded and contracted weights (in grams) of two *Polinices lewisi*.

Specimen no. 1			Specimen no. 2		
Time (*hr*)	Expanded	Contracted	Time (*hr*)	Expanded	Contracted
	290	192		340	197
2	240	187	8	290	180
7	300	197	12	310	201
14	261	196	14	250	200
24	292	189	24	320	205

The influx of "raw" sea water into the tissues of the snail poses problems. It is obvious the admixture of blood and sea water cannot be tolerated, neither can such a large mass consisting of a complex meshwork of muscular and neural fibres (Fig. 2D) be entirely unsupplied by blood vessels. The solution has been the ensheathing of all musular and neural structures with a collagen-reaction type connective tissue, protecting all tissues from direct contact with the seawater in the aquiferous sinuses (Fig. 2C).

The arterial supply to the foot is in its posterior portions homologous with other prosobranchs. Anteriorly the aorta passes below the radular pouch and divides into cephalic and pedal branches. After plunging into the foot the pedal aorta divides into posterior and anterior vessels which further divide and branch out to embrace all parts of the podial and metapodial complexes (Fig. 2F). The capillary bed is continuous with the perimuscular sheaths, so that muscles and neural structures are bathed in blood and isolated from the sea water in the aquiferous sinuses. The sheaths are connected to isolated lacunae, the remains of the pedal venous sinus (Fig 2E, *ps*) which join with the cephalic sinus. From the cephalic sinus springs the cephalic vein, running to the kidney through a muscular septum placed anteriorly to the cephalic sinus. This muscular septum acts as a valve isolating the body organs from the hydrostatic pressure of the foot.

FOOT EXPANSION AND MOVEMENT

The major portion of the expansion and oedematous condition of the foot is due to the uptake of surrounding water by way of the postpodial ostia. Blood pressure within the muscle sheaths probably does have some effect, especially in early phases of expansion. A contracted snail placed on damp weed will expand to a small degree, the edge of the postpodium being extended and capable of movement. This is entirely due to blood pressure in the muscular sheaths. If at this stage the edges of the postpodium are sealed with haemostats, the snail is unable to expand even if placed in water.

When a contracted snail is placed in water the postpodium is first extended and engorged with water, a sudden series of contractions expands the propodium and more water is now taken up by the postpodium. The water take-up is due to the relaxation of the muscles of the foot coupled with the tumescence of the perimuscular sheaths, which cause a lengthening of all muscle bundles and enlargement of the aquiferous sinuses. Contraction is the opposite action, the detumescence of the blood-containing sheaths and contraction of the muscle fibres. Figure 3 (*upper*) is a pressure graph of conditions in the propodium (P) and in the pericardial cavity (B). Pressure fluctuations are evident in the contracting foot, and the visceral pressure is uniform except for a rise on closure of the operculum (A on chart). This pressure differential is attributable to the muscular septum pierced by the cephalic vein.

A great degree of differential pressure control can be exercised by *P. lewisi*. This hydrostatic system is of importance for movement through the substrate. Although the snail is provided with a coating of cilia on all exposed surfaces, these are not used for progression. The snail spends most of the time partially or entirely buried in sand, mud, or gravel. Movement is accomplished by the thrusting forward of the propodium, then its dilation with water to push upwards and away the substrate, pulling the body and shell of the snail along. In the aquarium boulders up to 4 lb have been moved by travelling snails. The mechanics of movement are different from that displayed by many

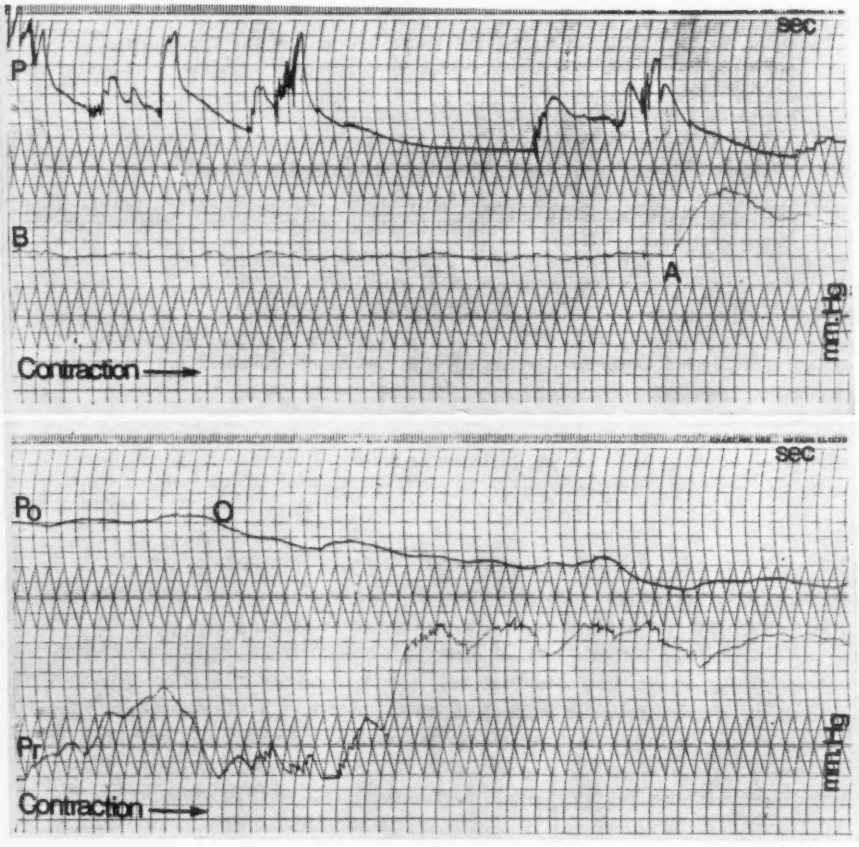

FIG. 3. Pressure graphs of contracting *Polinices lewisi*: *Upper*: P, pressure in propodium; B, body pressure; A, closure of operculum. *Lower*: Pr, pressure in propodium; Po, pressure in postpodium; O, ejection of water from ostia.

bivalves, where the distal end of the projected foot is expanded to form an anchor and the body pulled along by the contraction of the extended foot. Figure 3 (*lower*) demonstrates the pressure differential between the propodium and postpodium. Rapid contractions and pressure rises are apparent in the anterior portion, while there is a steady pressure rise in the postpodium, water starting to pour out of the ostia ("O" on chart).

ACKNOWLEDGMENT

Grateful thanks are extended to D. B. Quayle who suggested the problem and under whom this research was carried out.

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Growth and Spore Production of *Clostridium botulinum* Type E in Chemically Defined Media[1]

By G. A. Strasdine and Joanne Melville

Fisheries Research Board of Canada
Vancouver Laboratory, Vancouver, B.C.

ABSTRACT

A chemically defined medium is described for the growth and spore production of *Clostridium botulinum* type E, strain Minnesota. With the exception of valine, omitting any amino acid or vitamin did not prevent growth, although marked changes in morphology or reduced growth were usually evident. Choline was shown to be an essential factor in promoting cell division and was capable of exerting this action after maximum growth was attained. Spore development in the medium was markedly influenced by the source of available carbon and ranged from complete sporulation of cultures grown on maltose and glucose to complete inhibition with galactose.

INTRODUCTION

Interpretation of data obtained from studies on the cellular processes of spore formation and germination is frequently complicated by the complexity or uncertainty of the components present in the culture medium. This is particularly evident in media described for the growth of the anaerobic spore-forming organisms for which a balanced and often complex mixture of amino acids, vitamins, and various other growth factors is required. In addition, the media must establish a specific, often narrow, oxidation-reduction potential for growth initiation. The development of chemically defined media for the elucidation of the mechanisms of spore, toxin, or enzyme production in these organisms has consequently lagged behind similar studies in the less fastidious aerobic forms.

The development and use of defined media for the growth of *C. botulinum* have been reported by Perkins and Tsuji (1962) for type A, and more recently by Ward and Carroll (1966) for type E. These, in addition to other nutritional investigations (Day and Castilow, 1964), have augmented our knowledge and understanding of the growth of *C. botulinum* and at the same time have revealed new areas for research.

In previous studies (Strasdine, 1967; Strasdine and Kelly, 1967) we made use of the characteristic high degree of sporulation exhibited by the Minnesota strain of *C. botulinum* type E in trypticase–peptone medium for obtaining large spore yields. Early in these studies we realized the advantages of employing chemically defined media for growth and spore production and a series of investigations leading to the development of such a medium were subsequently undertaken.

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The defined medium described in the following report promotes good growth and maintains the high degree of sporulation characteristic of this strain.

METHODS

Stock cultures of *C. botulinum* type E, strain Minnesota, were maintained in the PE-2 medium of Folinazzo and Troy (1954). Trypticase–peptone medium (Schmidt et al., 1962) containing 0.4% final concentration of glucose (sterilized by filtration) was employed for spore production. The large scale batch production of spores was accomplished by means of an FD Bench-Scale BiO-kulture model fermentor (Fermentation Design Inc., Durham, Penn.) equipped with a 14-liter vessel. The operation of the fermentor and the harvesting and cleaning of spores were as previously described (Strasdine, 1967).

Vegetative growth and spore formation in defined media were followed spectrophotometrically (625 mμ) and microsopically. Photographs were made with a Wild M20 microscope equipped with phase attachment and photoautomat assembly. Turbidity measurements for growth were made directly in 14 × 100-mm screw-cap tubes containing 5 ml of the appropriate medium. For larger volumes of media, water-jacketed double-arm culture flasks (spinner-flasks, Bellco Glass Inc., Vineland, N.J.) were employed. The flasks were easily modified for sampling, gassing, or monitoring *p*H. Spore inocula were prepared from a washed suspension diluted in distilled water. A 1% inoculum gave a final concentration of 1×10^6 spores/ml of medium.

Requirements for individual components of the defined medium (Table I) were established by a minimum of three successive transfers. Tube assays

TABLE I. Chemically defined medium for growth and spore production of *C. botulinum* type E.

Component	Concn (*mg/100 ml*)	Component	Concn (*mg/100 ml*)
L-α-alanine	40	Biotin	0.001
L-arginine	23	Thiamine	0.040
L-cysteine	17	*p*-aminobenzoic acid	0.001
L-glutamic	12	Riboflavin	0.100
Glycine	12	Inositol	0.100
L-histidine	20	Calcium pantothenate	0.100
L-isoleucine	50	Folic acid	0.100
L-leucine	150	Niacinamide	0.100
L-lysine	125	Pyridoxal	0.100
L-methionine	6	Choline	1.000
L-phenylalanine	100		
L-proline	50	$FeSO_4 \cdot 7H_2O$	0.200
L-serine	100	$CaCl_2 \cdot 2H_2O$	0.200
L-threonine	100	$MnSO_4 \cdot H_2O$	1.000
L-tryptophane	5	$MgSO_4 \cdot 3H_2O$	4.000
L-valine	100	NaCl	0.200
		K_2HPO_4	100
Glucose	500	KH_2PO_4	100

were incubated at 30 C in an anaerobic incubator. Anaerobic conditions in flasks were obtained by gassing with oxygen-free nitrogen.

Amino acid analyses were made with a Beckman amino acid analyzer. Glucose was assayed with the glucostat reagent (Worthington Biochemical Corp., Freehold, N.J.). All chemicals were of reagent grade. Biochemicals were obtained from Mann Research Lab. Inc., New York, N.Y., and were of the highest purity obtainable.

Stock solutions of components were added either individually or as blocks. The medium was adjusted to *p*H 7.0, diluted to the required volume, and sterilized by filtration.

Culture filtrates were assayed for toxicity by injecting intraperitoneally 0.5-ml aliquots of filtrate dilutions into white mice. Three to five mice were used per dilution and the mice observed for 4 days. Results were expressed as minimal lethal dose (MLD) per ml of undiluted filtrate.

RESULTS

The growth curve for *C. botulinum* type E, strain Minnesota, in the complete medium is shown in Fig. 1. Spore formation was first evident at 21 hr incubation and virtually 100% of the spores were refractile (Fig. 2) at 24 hr. The decrease in glucose concentration of the medium parallelled the drop in *p*H. The maximum rate of both activities was coincident with the stage of logarithmic growth of the culture.

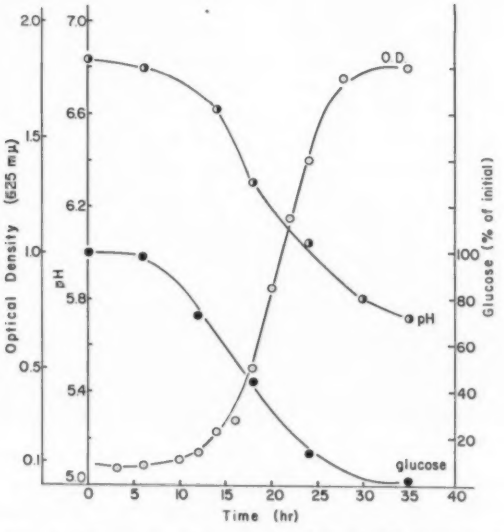

FIG. 1. Glucose utilization and *p*H change during growth of *C. botulinum* in the complete medium.

The concentrations of components shown in Table I allowed maximum growth. At one-half the concentrations shown, growth was reduced 25%. Increasing glucose concentration or maintaining a constant *p*H in the culture by means of a *p*H-stat had no effect on total growth or spore production although the amount of titratable acid produced was directly proportional to the initial glucose concentration.

Amino acid omission.—With the exception of valine we could not show an absolute requirement for a particular amino acid. The effect of omitting a single amino acid was most pronounced in tubes receiving spore inocula. Subsequent transfer of vegetative cells to identical medium shortened lag periods, increased total growth, allowed greater uniformity of vegetative cells, and synchronized sporulation. Valine omission retarded spore germination and completely inhibited spore outgrowth. The average O.D. values, expressed as per cent of the maximum O.D. (no omissions), obtained when amino acids were omitted singly from the medium were as follows: alanine, cysteine, phenylalanine, proline, 100; arginine, methionine, 95; tryptophane, glycine, 80; leucine, glutamic acid, 75; serine, 64; lysine, 60; threonine, 55; isoleucine, histidine, 50; valine, no growth.

Variations in cellular morphology were often very pronounced when amino acids were omitted singly, particularly in those cultures initiated from spore inocula. Three extreme examples of this are shown in Fig. 3–5 for cells growth in defined media in the absence of phenylalanine, serine, and leucine respectively.

Vitamin omission.—Spore germination was not inhibited by omission of any single vitamin. Maximum turbidity occurred when several vitamins were omitted singly from the medium, but typical cells were obtained only when all vitamins were present. Sporulation was markedly inhibited when folic acid or pyridoxal was omitted. In the absence of choline, cells became highly elongated and lost the ability to undergo normal division (Fig. 6). The addition of choline at a final concentration of 10 γ/ml to the 16 hr culture shown in Fig. 6 restored the culture to a more nearly normal form, giving only slightly elongated cells (Fig. 7). Acetylcholine, phosphocholine, or ethanolamine would not substitute for choline in the medium. The results of omitting vitamins singly on total growth and the observed effects on cellular morphology are described in Table II.

Salt omission.—When the various inorganic salts of the medium were omitted singly, cells remained generally typical, the main effect being a marked reduction in total growth:

Salt omitted:	None	$FeSO_4$	$CaCl_2$	NaCl	$MnSO_4$	$MgSO_4$	K_2HPO_4–KH_2PO_4
% maximum O.D.	100	77	30	29	23	21	18

Carbon source.—Variation of the carbon source of the medium had a marked effect on the sporulation process (Table III). Utilization of the carbon source with concurrent acid production was characteristic of those cultures giving good spore formation. Growth in the absence of acid production yielded

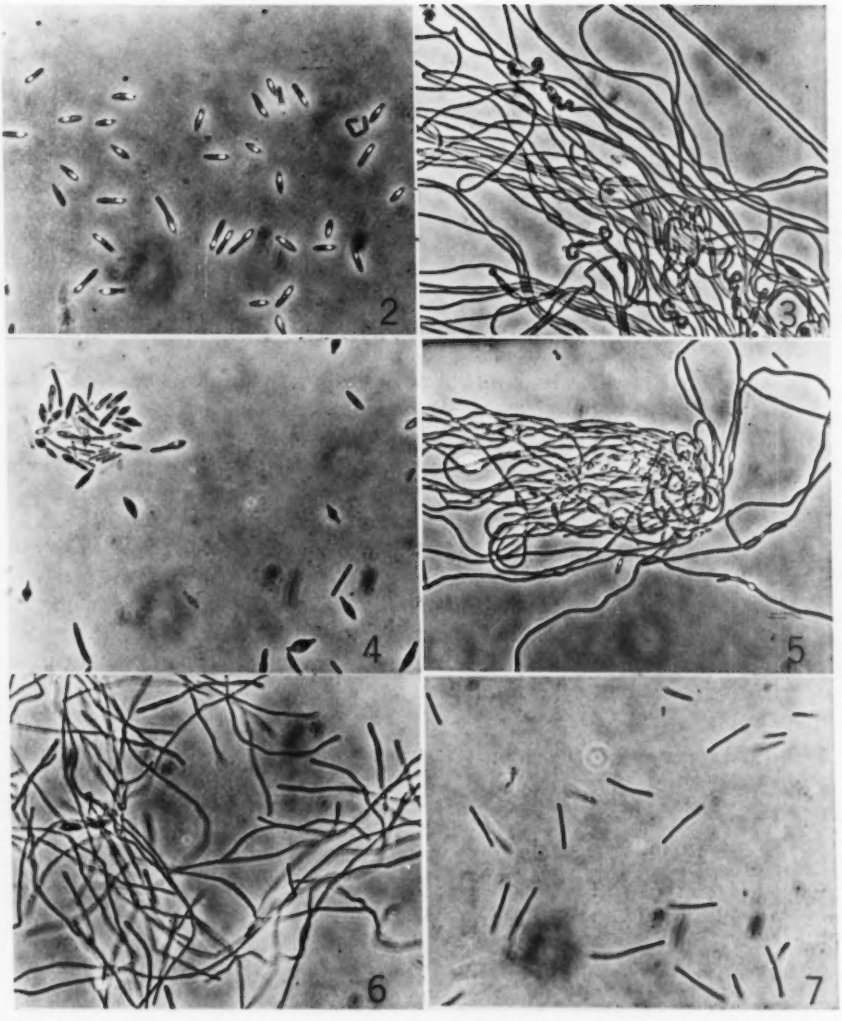

FIG. 2–7. Phase-contrast photomicrographs of *C. botulinum* type E in chemically defined media. 500×.

FIG. 2. Spore formation in complete medium.

FIG. 3–5. Effects of omitting phenylalanine, serine, and leucine respectively.

FIG. 6. The effect of omitting choline.

FIG. 7. Additional 24-hr incubation of culture shown in Fig. 5, after the addition of choline

TABLE II. Effects of omitting vitamins singly on total growth and cell morphology of *C. botulinum* type E in chemically defined media.

Omission	% maximum O.D.	Morphology
None	100	Typical
Inositol	100	Ends pointed
Riboflavin	100	Elongated & curved
Pantothenate	100	Elongated & curved
Folic acid	100	Slightly elongated; reduced sporulation
Biotin	95	Elongated, swollen
Pyridoxal	90	Swollen; reduced sporulation
Niacinamide	85	Elongated, swollen
Choline	65	Highly elongated, twisted
Thiamine	60	Typical
p-Aminobenzoic acid	45	Elongated, swollen

cells with a reduced ability to form spores. The most striking example of this was obtained with galactose as sole source of carbon and energy. Although 90% maximum growth was achieved, no spores were formed on this substrate. Subsequent experiments in which *p*H was maintained at, or adjusted to, 7.0 during growth did not alter the results.

Substitution of maltose for glucose in the medium increased total growth, allowed excellent sporulation, and reduced the length of the lag period.

Toxin production.—Culture filtrates from the Minnesota strain in trypticase-glucose-peptone medium characteristically contain from 1000 to 1500 mouse MLD/ml. All cultures showing growth in defined media were routinely examined for toxin production. Low levels of toxin (15–20 MLD/ml) were usually evident in cultures supporting good growth in defined media, i.e., at

TABLE III. Effects of various carbon sources on total growth of *C. botulinum* type E in defined media.

Carbon source	% maximum O.D.	Final *p*H	Morphology
Glucose	100	5.6	Typical
Fructose	50	6.3	Elongated
Galactose	90	7.2	No sporulation
Xylose	75	7.3	Elongated, sporulation reduced
Succinate	60	7.1	Elongated, sporulation reduced
Lactate	20	7.0	No sporulation
Acetate	50	6.9	Slow germination — no sporulation
Maltose	130	5.7	Typical
Lactose	70	7.0	Twisted, chains
None	–	7.0	No germination

least 80% maximum O.D. Omitting singly the various amino acids, vitamins or salts did not affect toxin production. Maximum titers were obtained after 3–5 days incubation.

DISCUSSION

The chemically defined medium permits rapid growth of *C. botulinum* type E, strain Minnesota, and maintains the high degree of sporulation characteristic of this particular strain.

As might be expected by the nature of the type E strains, little or no carbon or energy for growth was obtained when the defined medium contained amino acids as the sole source of available carbon. Amino acid analysis of the medium during various periods of growth, however, demonstrated a rapid disappearance of cysteine to about 50% of initial concentration in 16 hr. Unlike glucose utilization, however, cysteine utilization ceased at the onset of sporulation. This rapid disappearance was attributed to an active intracellular cysteine desulfhydrase enzyme system. The importance of this system to growth, spore formation, and germination is currently being investigated. There did not appear to be any energy gain from the pyruvic acid formed by the action of the enzyme on the amino acid.

The experiments in which amino acids or vitamins were omitted singly direct attention to specific areas where further studies might prove valuable. The apparent requirement for valine in initiating spore outgrowth and the rapid utilization of cysteine in substrate amount are interesting from the standpoint of growth suppression by means of antimetabolites.

Ward and Carroll (1966) reported no single amino acid requirement in their defined medium for the growth of the D8 strain, although omission of valine, tryptophane, or aspartic acid was detrimental to growth. The possibility of differences in the nutritional requirements of individual strains might explain the difference in observations. In accordance with their findings, we could not show an absolute requirement for any single vitamin.

The effect of choline on cell division of *C. botulinum* type E has recently been reported by Gullmar and Molin (1967). Inhibited chain formation and the appearance of rounded cell forms led these workers to suggest that choline exerts its effect on cell wall synthesis and cell division. The observations described above agree with this conclusion. The ability of choline to exert its effect after maximum growth is obtained suggests a more definite role for choline in cell division.

In general it appears that the process of cell division in *C. botulinum* type E is very susceptible to nutrient imbalances or deficiences. In the majority of instances the first and most noticeable effect of an inadequate medium was an abnormal or inhibited cell division; this was particularly evident with amino acid and vitamin omissions. In contrast, sporulation and motility were rarely affected and even the more irregular forms displayed both properties.

The increased growth with maltose as source of carbon and energy might be explained by the conservation of the energy released on disaccharide hydro-

lysis by means of a transglucosidation process. In this respect, we have observed an increase in the concentration of an intracellular, endogenously active, starch-like polysaccharide from maltose-grown cells. The importance of this material as an intracellular energy storage compound and its possible role in sporulation are both currently being studied.

Sporulation did not occur when cells were grown on galactose as the carbon and energy source. Whether this reflects the absence of specific enzymes with galactose, which are present and related to sporulation in cells grown in glucose, has as yet not been determined.

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Analysis of the Southern Gulf of St. Lawrence Cod Population[1]

By J. E. Paloheimo[2]

Fisheries Research Board of Canada
Marine Ecology Laboratory, Bedford Institute, Dartmouth, N.S.

AND

A. C. Kohler

Fisheries Research Board of Canada
Biological Station, St. Andrews, N.B.

ABSTRACT

The Gulf of St. Lawrence cod stock supports a fishery with recent annual landings around 65,000 metric tons. Traditionally, fish have been taken by baited hooks, but beginning in 1947 trawl fishing has gradually become the major fishing method.

Introduction of trawling with concurrent increase in landings from an average of 30,000 metric tons prior to 1947 to 65,000 metric tons since then appears to have greatly changed the composition of the stock. The changes were characterized by the lowering of the average size of fish and of the abundance in weight of the commercially fished sizes, and the increasing of the abundance of the pre-commercial sizes.

Available statistics permit estimation of growth, recruitment, and productivity parameters for the stock. Analysis suggests an inverse relationship between stock density and both growth and recruitment, the overall effect being an increased productivity.

If the observed population changes are responses to increased fishing, then per-recruit models are not appropriate to predict the effects of any past or future regulations. Models providing for differential growth efficiencies, and competition and food-related recruitment relationship are required.

THE FISHERY

Cod have been fished in ice-free months in the southern Gulf of St. Lawrence for more than a century. Up to 1947 the method of fishing was by baited hook or jigger but at that time a major change took place with the introduction of otter trawling by Canadians. This change, plus additional fishing by European otter trawlers beginning in 1952, had a marked effect on the population in the area.

Tagging experiments (McCracken, 1959; Martin and Jean, 1964) showed that the Gulf of St. Lawrence cod population is a migratory stock. The general pattern of migration is shown diagrammatically in Fig. 1. There are two main areas where the fishing appear to congregate and where the fishery on them

[1]Received for publication December 15, 1967.
[2]Present address: Department of Zoology and Department of Mathematics, University of Toronto, Toronto, Ont.

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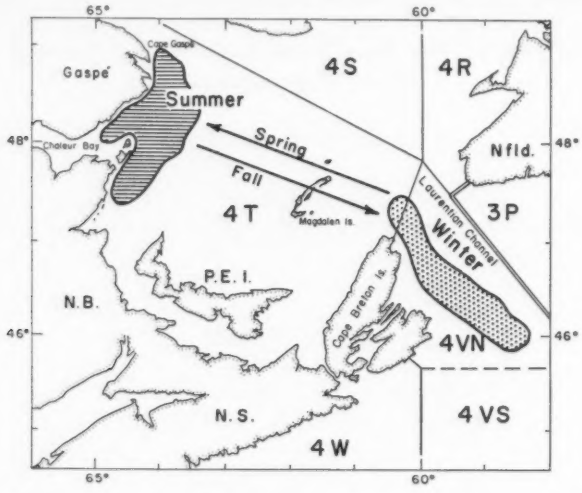

FIG. 1. Migrations of the southern Gulf of St. Lawrence cod stock, showing ICNAF statistical divisions.

is concentrated: first, the Magdalen Shallows area between the Magdalen Islands and Cape Gaspé–Bay Chaleur; and second, the edge of the Laurentian Channel off the northeast coast of Cape Breton. The annual migratory pattern is from the Magdalen Shallows area in the fall to the area of winter concentration near Cape Breton and back again to the Magdalen Shallows area in the spring.

Research-vessel surveys, using an otter trawl having a small-mesh lining in the codend, indicate that the migratory habit is more pronounced in older fish. This is reflected in differences in size and age composition in the winter and summer areas. The samples taken represent both pre-commercial and commercial sizes and ages to be found in the area. The differences are represented in Fig. 2. The upper part of the figure shows samples taken in the Cape Breton area in winter. When they are compared with those in the lower part of the figure (from the southwestern Gulf), it can be seen that the former group is composed of longer, older fish.

Fishing fleets have taken advantage of these migrations and concentrations, particularly during the last 15 years. The Canadian otter-trawl fleet tends to concentrate its fishing activity on the stock when it is in the Magdalen Shallows area in the summer whereas other nations fish it when the cod are concentrated along the edge of the Laurentian Channel north of Cape Breton in winter.

Annual landings of cod from this population by Canada and other countries are shown in Fig. 3. Early statistics for the years 1936–51 show a wholly Canadian catch, fluctuating between about 20,000 and 53,000 metric tons.

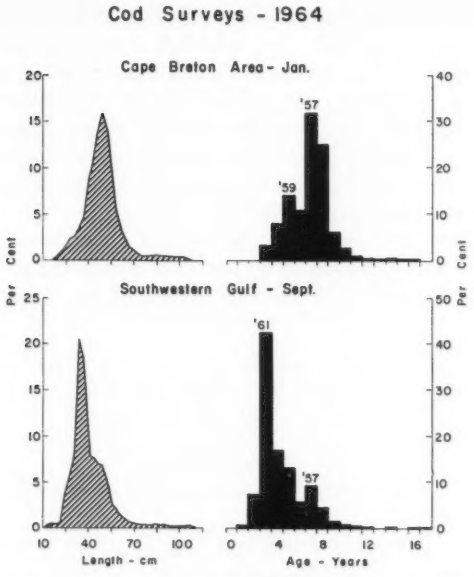

FIG. 2. Lengths and ages of ICNAF Div. 4T cod caught by a research vessel.

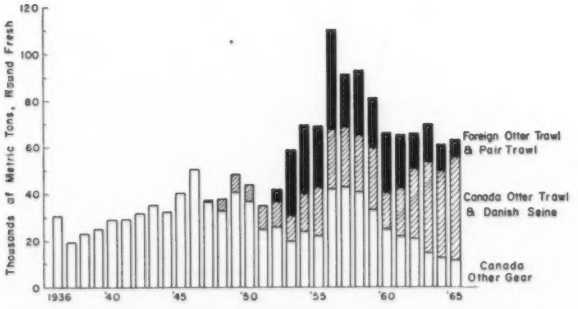

FIG. 3. Annual landings of southern Gulf of St. Lawrence cod; 1952–53 landings are yearly totals for Div. 4V; 1954–57 landings are 1st quarter Div. 4Vn and 4Vs; 1958–59 landings are 1st quarter Div. 4Vn and 4V (4Vs not included); 1960–65 landings are 1st quarter 4Vn only; all Div. 4T landings are annual.

The introduction of otter trawling and the activities of other countries led to a rapid rise in landings to a peak in 1956 of 110,000 metric tons. The total has since decreased and seems to be levelling off at somewhere between 60,000 and 65,000 metric tons, partly on account of decreases in landings by France, Portugal, and Spain during the 1960–65 period.

Canadian landings peaked at about 69,000 metric tons in 1957, dropped fairly rapidly to 40,000 metric tons in 1960, and increased again to about 56,000 metric tons in 1965. Since its introduction, otter trawling has become progressively more important, and this is particularly noticeable in the figure from 1956 onwards. Since that time, landings by other Canadian gears, mainly various types of line fishing, decreased each year. It should be noted in the figure that, although otter trawling and Danish seining are combined, Danish seining still provides less than 5% of the total.

During the last 15 years there have been changes in the size and age composition of the cod population. Figure 4 shows size composition of landings of cod for the 1949–51, 1956–57, and 1962–65 periods. In earlier years (1949–51),

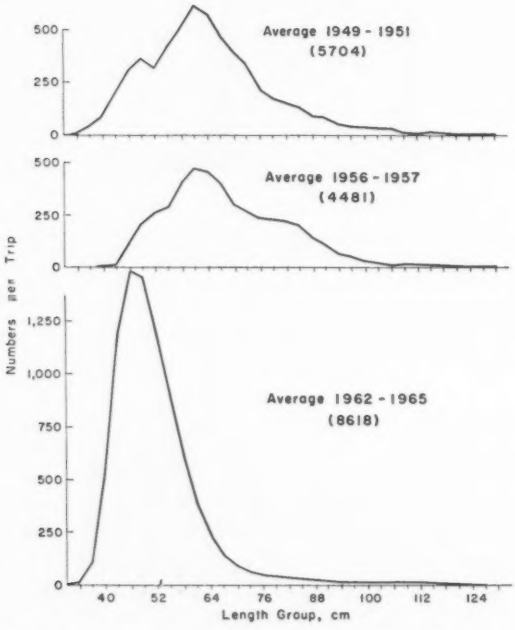

FIG. 4. Length frequencies of ICNAF Div. 4T cod in numbers per trip.

a group of fish was being landed with a peak size at 61 cm. This is shown in the top of the figure. During 1956–57 the mean size landed was particularly high on account of accelerated growth. More recent years are shown in the bottom of the figure, with an average for 1962–65. It is apparent that the bulk of the fish landed recently are between 37 and 70 cm in length, a usual peak size being at about 46 cm.

Comparable age compositions are shown in Fig. 5. For the earlier years and during the fast-growth period the dominant age-group was 7-year-olds. Also, there were fair numbers of fish landed that were 10 years of age or older, particularly in 1949–51. The figure shows that during more recent years the usually dominant age-group has been 6-year-olds. Separate data not shown

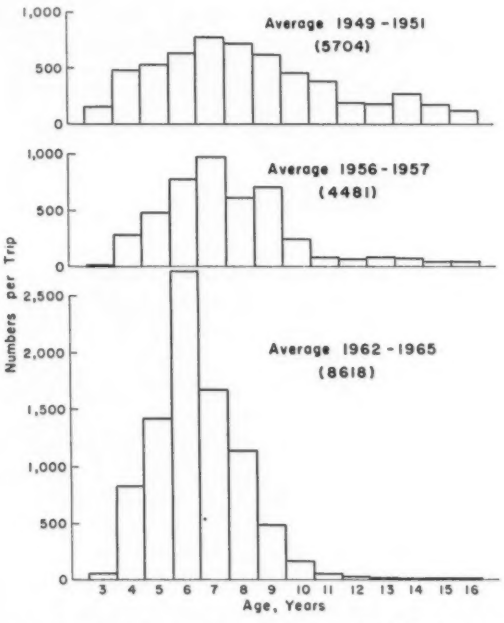

FIG. 5. Average age composition of ICNAF Div. 4T cod stock.

for 1965–66 show the catch to be predominantly 4-year-olds. The number of fish over 10 years of age in the landings has decreased markedly. Both Fig. 4 and 5 were calculated on a numbers-of-fish-per-trip basis for Gloucester-class draggers (26–50 tons). The numbers in brackets in Fig. 4 and 5 giving catch per trip in numbers for the three periods provide an interesting comparison. There were less fish per trip landed from the years in which the fish were larger and older than in recent years in which the fish were smaller and younger.

The change in size composition of the catch has affected the numbers of fish discarded at sea by fishermen. Trips to sea by staff of the St. Andrews Biological Station have helped to keep track of the changes in discard practices. Data on discards at sea are shown in Table I. Their numbers have decreased yearly since 1956. Initially high discards were due partially to the small mesh being used by these draggers, but after 1959, when mesh regulation

TABLE I. Average discards at sea of cod catches, ICNAF Div. 4T.

Year:	1956	1957	1958	1959	1960	1961	1962	1963	1964	1965
Avg discarded (numbers) (%)	22.9	21.1	16.9	10.4	10.5	4.6	3.0	3.0	1.6	1.0
Mesh size (*inches*)	3	3–4½	4–4½	4½–4⅞	4⅜–4½	4⅜	–	4½	4⅛	4⅛–4⅝

Age:	2 & under	3	4	5	6
Avg discarded by age 1949–56 (%)	18	39	40	3	0

became strictly enforced, the annual decrease in discards continued. This was due mainly to a gradual lowering of sizes accepted at fish plants handling the catch from the Div. 4T cod population as the availability of large cod went down. Average age composition of discards for 1949–56 is shown in the bottom panel of Table I. It shows that discards were under age 6, and this has continued to be the case in subsequent years.

Change in availability of fish is reflected in landings-per-unit-effort data in Fig. 6. Long-term data for the Canadian otter-trawl fleet are available since the start of dragging by Canadians. The overall trend from 1948 to 1964 for Canada is a downward one, although a low was reached in 1960 and since then there has been an increase. Catch-per-unit-effort data for Portugal and Spain are also available from ICNAF Statistical Bulletins and follow a similar trend from 1954 to 1960, but take a marked upswing from that time until 1963. The indication is that this fleet now fishes the population only at a time when it is most densely concentrated in the Cape Breton area, and this is borne out by the total decrease in foreign landings shown in Fig. 3.

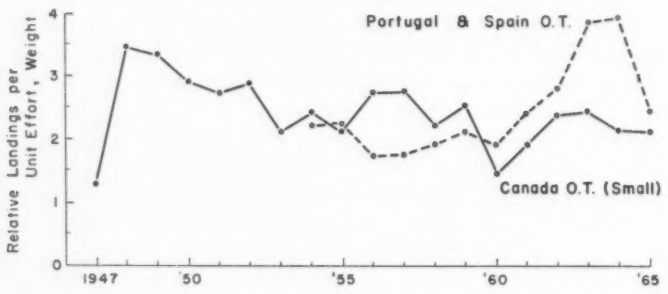

FIG. 6. Landings per unit effort of ICNAF Div. 4T cod.

Catch per trip for survey cruises are compared with those for commercial vessels in Fig. 7. It may be noted in Fig. 7 that surveys carried out by the St. Andrews Biological Station on an annual basis on the cod population in

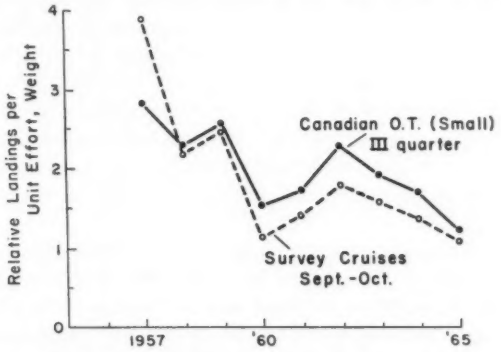

FIG. 7. Relative landings per unit effort for commercial and survey cruises from the ICNAF Div. 4T cod stock.

the Magdalen Shallows area produce abundance trends which are very similar to those taken from commercial otter-trawl landings. The survey is undertaken each fall by a vessel similar in size to most of those used by the northern New Brunswick otter-trawl fleet, using a net with a small-mesh liner ($1\frac{1}{2}$ inch) in the codend. The data provide a forecast of availability and sizes for the following year. The high value obtained for 1957 may be unrealistic due to a conversion factor used for the survey by a smaller boat during that year (Jean, 1964). However, trends in availability from the two sources are very similar from 1958 on.

As mentioned earlier, a further factor affecting sizes and ages of cod landings from the Gulf stock has been changes in growth. This is illustrated in Fig. 8 where mean sizes of age-groups from 6 to 10 have been plotted for

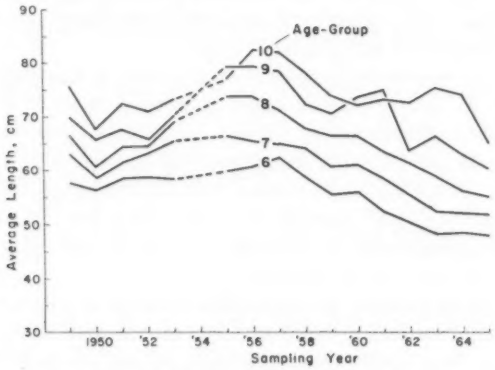

FIG. 8. ICNAF Div. 4T cod age-length data.

1949–65. The data were taken from commercial landings. A peak size-at-age is shown in the 1956–57 period and was mentioned previously; this was due to a marked increase in growth. The main reason for this increase appeared to be readily available food due to a mass herring mortality (Kohler, 1964). More recent age/length data show that average size-at-age has been dropping steadily since the fast-growth period and the lack of larger fish in the landings has made averages for 9- and 10-year-olds less reliable. The figure indicates some evidence of levelling off of 6-, 7-, and 8-year-olds at their present growth rate and, although this is not as high a mean length-at-age as was found in the 1949–53 period, the spacing of the points for the various age-groups indicates that the rate of growth of commercial-size fish is about as fast if not faster than in 1949–53.

Studies carried out during surveys since 1959 have indicated some changes in volume and species of food taken by these fish, which may be related to the growth change. In addition, average size and age at maturity show fluctuations from year to year, with some tendency toward lower values recently.

USE OF DATA

In the foregoing section and in those that follow, certain statistics of the fishery have been used and some explanation of the manipulation of them is warranted. The length and age sampling of commercial landings was carried out by technicians from the St. Andrews Biological Station. A variety of size categories are sorted and landed, and these have to be weighted in the samples according to the total landings of the size category of that particular gear during the year. Data for 1954 commercial landings and some for 1953 were not used in this study because of inadequacies in weighting except in some time series where they were essential and had to be interpolated. Seasonal age and length compositions were combined by weighting them according to total landings per season to give a yearly frequency to arrive at an average length composition per season. Fish measured for length were then further sampled for age to arrive at an age/length key. Application of the age/length key to seasonal length frequencies gave us then the age composition of landings per season and per year. Table II lists the estimated age composition for 1949–65 on a number-of-fish-per-trip basis.

Because of the migration pattern shown in Fig. 1, landings from the Cape Breton area had to be included in order to show a picture of the take from the total population after trawling started (Fig. 3). This was done by adding landings for Div. 4V for 1952–53, by adding first quarter 4Vn and 4V (4Vs was not included) for 1958–59, and by adding first quarter 4Vn only from 1960 on. These were added to all 4T annual landings. This last breakdown is given for the 1960 period onwards only because prior to that 4Vn data were not available separately in ICNAF statistics.

Catch-per-unit-effort data for Canadian otter trawlers were obtained from a log and interview system carried out by technicians of the St. Andrews Biological Station. Most of the Canadian boats fishing the Gulf area and landing

TABLE II. Cod catches in numbers per trip, using otter-trawl, pair-trawl, and Danish-seine data. ICNAF Div. 4T.

	Age																
Year	4[a]	5[a]	3	4	5	6	7	8	9	10	11	12	13	14	15	16+	
1949	715	–	222	292	433	675	1002	1123	491	537	340	160	244	164	10	25	5718
1950	826	385	149	387	353	592	859	474	976	300	484	207	79	493	382	171	5906
1951	1185	844	91	775	813	773	475	512	376	561	361	216	197	118	116	109	5493
1952	1149	2009	48	669	1974	901	472	465	399	373	522	264	158	49	48	105	6447
1953	395	749	14	69	719	1215	452	235	302	205	320	460	143	87	69	71	4361
1954	919	525	58	565	436	2046	1634	320	84	466	355	115	207	82	75	–	5457
1955	747	1880	49	413	1859	447	773	526	158	57	60	68	45	21	12	15	4503
1956	522	330	–	199	307	1265	557	790	687	145	75	51	116	90	33	44	4359
1957	935	769	9	385	666	320	1394	432	722	350	79	66	52	56	40	34	4605
1958	1163	1970	–	656	1932	912	304	969	218	206	116	47	12	12	2	5	5391
1959	835	3172	23	430	3137	2432	1022	258	517	128	109	48	6	2	15	–	8127
1960	930	1201	22	699	1186	1379	807	203	71	123	30	27	7	2	2	–	4558
1961	764	2542	–	621	2536	1719	1378	459	75	37	76	39	13	6	3	1	6963
1962	464	2724	4	463	2717	4263	1059	555	217	45	8	14	2	1	1	1	9350
1963	586	920	38	588	923	3783	2662	907	362	85	47	10	18	2	1	2	9428
1964	–	920	203	1247	919	951	2329	1437	498	191	60	20	8	11	3	3	7880
1965	–	–	38	1032	1134	1894	678	1619	864	358	106	62	20	1	5	6	7817

[a]Discards added to landings.

in the past years in the Maritime Provinces are between 25 and 75 gross tons and of Gloucester-class construction. Furthermore, the pattern of fishing has been fairly similar from year to year. Studies have shown that the length of their trips is usually a week and that this varies little over the course of the fishing season. Since there has been no appreciable change in the average length of time of a trip, we have taken number of trips as the measure of effort for the Gloucester-class boats.

The catch per trip for Gloucester-class boats has been tabulated in Table III, column 5. The total landings from the stock have then been divided by the catch-per-trip figure for Gloucester-class boats to arrive at an estimated number of trips per year as if all the landings had been made by boats in this class. Details of the calculations are shown in Table III.

MORTALITY RATES

Catches per effort at age (Table II) and estimated total efforts (Table III) may be used to calculate mortality rates. For that purpose we put: i, subscript referring to age; j, subscript referring to year; ${}_iC_j$, catch; f_j, relative effort; q, catchability coefficient; M, instantaneous natural mortality rate; F_j, instantaneous fishing mortality rate; ${}_iZ_j$, total instantaneous mortality rate.

Making the customary assumptions that the instantaneous fishing mortality rate is proportional to effort, i.e.

$$F_j = qf_j$$

and that the natural mortality rate is constant, we may put (Paloheimo, 1961)

$$ {}_iZ_j = \log \frac{{}_iC_j/f_j}{{}_{i+1}C_{j+1}/f_{j+1}} = q(\tfrac{1}{2})\ (f_j+f_{j+1}) + M \qquad \ldots\ldots(1).$$

Table IV gives the values of total mortality rates ${}_iZ_j$ for pairs of age groups 6/7, 7/8, 8/9, and 9/10, as well as the mean efforts $\frac{1}{2}(f_j+f_{j+1}) = \bar{f}_j$.

To determine whether the instantaneous mortality rate is dependent on effort f_j as suggested by equation 1, a covariance analysis has been carried out on values given in Table IV. The results are exhibited in Table V. The table shows that the regression of ${}_iZ_j$ on $\bar{f}_j$ is not significant for any of the pairs of age-groups and, in fact, the value of the regression coefficient, q, in equation 1, has a negative sign for ages 7/6 although it is not significantly different from zero.

To confirm the results of the covariance analysis the data in Table III were divided into three groups: the earlier years, 1949–52, when total fishing effort was less than 3000 units; the transitory period 1954–55; and the later years, 1956–65, when effort was mostly above 6000 units. Mortality data pertaining to the first and third groups were then subjected to analysis of variance to see if data would indicate any significant difference between the

TABLE III. Calculations of relative catch per effort and total effort figures.

Year	Catch per unit effort 1st & 2nd quarters	Catch fraction 1st & 2nd quarters	Catch per unit effort 3rd & 4th quarters	Catch fraction 3rd & 4th quarters	Weighted catch per unit effort	Relative units of effort		
						Total all gears	Total otter trawl, pair trawl, Danish seine	Canadian landings only
1948	1.77	0.50	1.69	0.50	1.74	2198	291	2198
1949	1.74	0.50	1.61	0.50	1.68	2883	440	2883
1950	1.62	0.46	1.31	0.54	1.46	3015	442	3015
1951	1.48	0.46	1.30	0.54	1.38	2524	722	2524
1952	1.70	0.47	1.22	0.53	1.45	2893	1082	2530
1953	1.23	0.44	0.95	0.56	1.07	5506	3621	2958
1954	0.65	0.27	1.43	0.73	1.22	5752	3794	3393
1955	0.80	0.32	1.19	0.68	1.07	6462	4387	4012
1956	0.97	0.32	1.58	0.68	1.38	8004	4970	4959
1957	1.24	0.33	1.43	0.67	1.37	6620	3472	5032
1958	1.06	0.41	1.15	0.59	1.11	8338	4752	5990
1959	1.30	0.45	1.28	0.55	1.29	6276	3679	4664
1960	0.69	0.46	0.78	0.54	0.74	8973	5574	5467
1961	1.06	0.49	0.86	0.51	0.96	6831	4534	4422
1962	1.25	0.49	1.15	0.51	1.20	5528	3730	4298
1963	1.46	0.56	0.96	0.44	1.24	5661	4232	4328
1964	1.25	0.59	0.86	0.41	1.09	5555	4340	4587
1965	1.31	0.63	0.62	0.37	1.06	5946	4815	5315

TABLE IV. Estimated mortality rates at age and mean efforts.

Year	Mortality at age 7/6 x_1	7/8 x_2	8/9 x_3	9/10 x_4	Mean relative effort
1949–50	+.1757	−.8209	−.1347	−.5727	2949
1950–51	−.2268	−.5225	−.2869	−.7403	2769
1951–52	−.4573	+.0048	−.2692	−.0202	2708
1952–53	−.6235	−.5605	−.4004	−.5310	4200
1953–54	+.1458	−.5024	−1.2379	+.3088	5629
1954–55	−.8096	−.9707	−.5413	−.2194	6007
1955–56	+.2611	+.0184	+.2438	−.1064	7233
1956–57	+.0928	−.3424	−.1720	−.7074	7312
1957–58	−.0693	−.3886	−.6367	−1.1647	7479
1958–59	+.1112	−.1603	−.6275	−.4568	7307
1959–60	−1.1147	−1.6095	−1.2837	−1.4398	7624
1960–61	−.0090	−.5726	−1.0051	−.6387	7902
1961–62	−.4861	−.9088	−.7424	−.4893	6180
1962–63	−.4732	−.1531	−.4232	−.9365	5595
1963–64	−.4976	−.6180	−.6033	−.6311	5608
1964–65	−.3369	−.3638	−.5126	−.3355	5750

TABLE V. Analysis of covariance of mortalities against mean fishing efforts (data from Table IV).

Age	Mortality sum of squares	Gross product	Effort sum of squares	Regression coefficient q	Sum of squares due to regression	Residual sum of squares
7/6	2.34970	+ 7.96926	4688.6828	−.001699	.005881	1.014581
8/7	2.54741	−13.01654	4688.6828	+.002776	.015692	1.090633
9/8	2.40373	−39.02843	4688.6828	+.008324	.141086	0.902839
10/9	2.77592	−32.95006	4688.6828	+.007027	.100563	1.105004
10/6	1.41974	−19.25932	4688.6828	+.004108	.034359	0.582228

		Based on Table IV	Based on catch curve
Mean total mortality rates: (age 6–10)	1949–51	Z = 0.39	Z = 0.12
	49–52	Z = 0.32	Z = 0.12
	55–65	Z = 0.51	Z = 0.59
	62–65		Z = 0.67

two groups, one representing a period of low and the other a period of high fishing effort. The results are shown in the bottom panel of Table VI and do not show statistically significant differences in the mortality rates between the two periods.

TABLE VI. Analysis of variance of mortality rates given in Table IV.

	Sum of squares	df	Mean sum of squares	F
Total	8.884	51		
Years	5.466	12	0.455	7.00*
Ages	1.095	3	0.365	5.62*
Within years	2.323	36	0.065	
Total	5.466	12		
Between 1949–52 1955–65	0.315	1	0.315	0.67
Within	5.151	11	0.468	

*Significance at 5% level.

Although statistically not different, the mean values Z = 0.32 and Z = 0.51 for 1949–52 and 1955–65 differ appreciably. Using these average values of Z and mean effort figures for the same period in equation 1 we get

$$0.32 = M + q\ 2809$$
$$0.51 = M + q\ 6799.$$

Solving the above for q and M we get M = 0.19 and q = 0.000048 and hence F = 0.13 ($=q\bar{f}$) for 1949–52 and F = 0.32 for 1955–65. These values for fishing mortality rate are somewhat too low to be consistent with the appreciable differences in the age and length compositions observed since the introduction of trawl fishing.

Estimates of total mortality rates by use of equation 1 and Table IV may be contrasted with the estimates of total mortality rates from the catch curve. By calculating an average number of fish caught per trip at age for the periods 1949–52 and 1955–65 and estimating the average decline of the abundance at age, we get the estimates of the total mortality shown at the bottom of Table V, ranging from Z = 0.12 to Z = 0.67. The estimates are obtained from the slope of line giving best fit to logarithms of the average catches per trip at age.

Although the total mortality figures based on a catch curve are rather unreliable since they reflect not only the mortality rate but also any trend or fluctuation in the recruitment, the contrast between the two sets of mortality figures, one showing a marked influence of fishing on the stock and the other a less evident effect, calls for an explanation.

Returning to mortality estimates based on catch and effort data, we recall the analysis of variance of total mortality rates shown in Table VI. Although the covariance analysis failed to show a significant regression of total mortality on effort, the analysis of variance indicates significant differences between years. We thus conclude that there are year-to-year variations in

the catchability, q, or natural mortality, M, which are greater than trends in the total mortality due to increased fishing.

Table VI also shows that there are significant differences between pairs of age-groups in the total apparent mortality rate. This could be attributable either to changes in q or M with age. Assuming different q and M at each age, equation 1 now takes the following form:

$$ {}_iZ_j = \log q_i/q_{i+1} + \tfrac{1}{2}q_i f_j + \tfrac{1}{2}q_{i+1}f_{j+1} + \tfrac{1}{2}(M_i + M_{i+1}) \quad \ldots\ldots(2). $$

Summing equation 2 over all years i and putting approximately

$$ \sum_1^{n-1} f_j \sim \sum_1^{n-1} f_{j+1} $$

we get

$$ \frac{1}{n-1}\Sigma\, {}_iZ_j = \log q_i/q_{i+1} + \frac{q_i + q_{i+1}}{2}\,\frac{\Sigma f_j}{n-1} + \tfrac{1}{2}(M_i + M_{i+1}). $$

The last two terms represent the average mortality at ages i and $i+1$ and the first term, $\log q_i/q_{i+1}$, represents a bias in the estimate.

If the catchability is increasing with age, i.e. if $q_i < q_{i+1}$, as suggested by figures in Table IV, then the bias term, $\log q_i/q_{i+1}$, takes a negative value and we thus get an underestimate of the mean mortality rate for the age-groups. This could account for some of the differences in the mortality estimates based on catch-effort on the one hand and catch-curve on the other.

Since our studies have indicated that the catchability coefficient, q, and possibly the natural mortality rate, M, change both with age of fish and year, a more specific model than equation 1 incorporating these changes was developed. Let

a_i = relative catchability of the i^{th} age-group
q_j = catchability in the year j

where we may take, say $a_9 = 1$ as a base line, then equation 1 may be written as

$$ {}_iZ_j = \log a_i q_j/a_{i+1}q_{j+1} + M + \tfrac{1}{2}a_i q_j f_j + \tfrac{1}{2}a_{i+1}q_{j+1}f_{j+1} \quad \ldots\ldots(3). $$

The parameters a_i, q_j, and M in equation 3 could be estimated by first obtaining approximate values for them and then calculating least squares corrections for the initial estimates by linearizing equation 3 for the corrections. To obtain meaningful values it would be essential that most correlation coefficients between, say, ${}_iZ_j$ and ${}_kZ_j$ over years j, and ${}_iZ_j$ and ${}_iZ_l$ over ages i are significant. Some sample values are listed below. The listed values of the correlation coefficient fluctuate widely, hence no further attempts to arrive at specific values of q and M were made.

Correlation coefficients between years and ages are as follows:

Between years: Years 1949–50 and 1950–51 marked as 1950, etc.

	1950	'51	'52	'53	'54	'55	'56	'57	'58	'59	'60	'61	'62	'63	'64
r =	.80	−.88	.14	−.79	.22	.41	.94	.94	.91	.26	.42	.48	−.80	.16	.22

Between ages:

	7/6 & 8/7	8/7 & 9/8	9/8 & 10/9
r =	.60	.57	.18

It is rather surprising that the effect of a twofold increase in effort cannot be demonstrated in our mortality estimates. Although the sampling error is probably quite large, one suspects that it does not account for all the variabiilty in the data. We expect in fact that as an important addition to sampling error, our failure to estimate the components of the total mortality arises from deviations from the basic equations 1 or 3 and possibly also from compensations by the fish stock to changes in the amount of fishing effort. These are examined in more detail in the next sections.

CHANGES IN FISH STOCK

Recruitment

The increased fishing on the Gulf of St. Lawrence cod stock has resulted in increased landings and apparently in concomitant changes in ages and lengths landed. Such changes in ages and lengths landed are expected on the basis of catch-per-recruit-models. However, there have been other changes in this fish population which are not so readily predictable.

In Fig. 9 we have plotted the catch per trip of 6- and 7-year-old cod. The figures given are 3-year running averages with midpoints ranging from 1950 to 1964. Regarding the catch per unit of effort of 6-year-olds as indicative

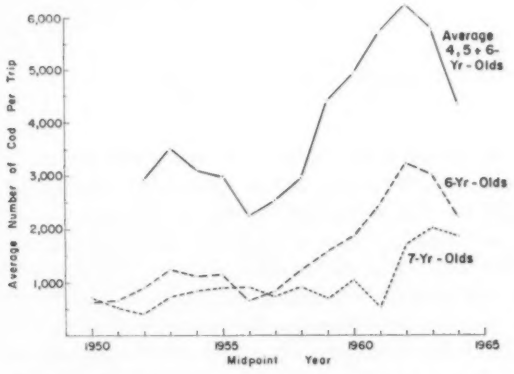

FIG. 9. Three-year running average of catch per effort in numbers for ages 6, 7, and ages 4, 5, and 6 combined.

of their abundance, Fig. 9 suggests that recruitment to the fishery at age 6 has increased since about 1959, that is, 1953+ year-classes have been more abundant in general than year-classes prior to 1953.

The abundance of a year-class at age 6 not only reflects the initial abundance of the year-class but also the amount of fish taken from it at earlier ages. Since prior to the introduction of a larger mesh size in 1957–58, more fish were presumably taken at younger ages and discarded than after the change, the trend in the recruitment observed in Fig. 9 might be an artifact or at least somewhat exaggerated. Because of lack of information on the mortality rates at younger ages, we cannot estimate the effect of removal of younger fish by the fishery on the abundance figures at age 6. However, to some degree the effect would be minimized if we calculate the average catch per trip at ages 4, 5, and 6, and average them for each year-class. This has been done by using discard information for 1956, increasing the figures giving the numbers at each age landed proportionately, dividing them by numbers of effort units, and totalling the resulting figures by year-classes.

The average catches per trip for combined ages 4, 5, and 6 by year-classes have been plotted in Fig. 9 as well. Each line in Fig. 9 demonstrates an upward trend in recruitment. Since the graphs are based on average catch-per-effort figures, we expect the actual upward trend to be even greater than shown in the figure; that is, to relate catch-per-effort figures to the abundance the figures should be multiplied by a factor ${}_iZ_j/(1 - \exp - {}_iZ_j)$. This multiplier increases with the fishing mortality or effort. Hence application of this correction would make the upward trend even more pronounced than shown in Fig. 9.

Since the catch-per-effort figures are subject to year-to-year variations in catchability and to sampling errors, we have also calculated virtual population size estimates following the method of Paloheimo (1958). This method assumes that we know the catches and both fishing and natural mortalities. The method is simple and based on the catch equation written in the following form:

$$\frac{F_j+M}{F_j} C_j + N_j = N_{j+1}$$

where N_j, N_{j+1} is the population size of a year-class at the beginning of the year j, $j+1$, and C_j is the catch from it. Hence, by adding up all the annual catches multiplied by the correction factor, F_j+M/F_j, from a year-class at and after age 6, we thus arrive at the estimated population size at the beginning of age 6. The correction factor with which catches are to be multiplied is the inverse of the fraction the fishing mortality is of the total.

The virtual population estimates have been calculated using $M = 0.19$ and $q = 0.000048$. Table VII gives the values of the population size estimates and the 3-year running averages have been plotted in Fig. 10. They confirm the general upward trend in the recruitment. We note that the last value for

TABLE VII. Virtual population size estimates at age 6.

Year	Summation for age 6 and over x	Summation up to age 10 y	y/x %
1949	7609.31		
1950	7881.23		
1951	5447.22		
1952	9004.37		
1953	27854.88		
1954	26939.81	26004.15	96.5
1955	11762.44	11181.17	95.0
1956	26962.21	26146.95	97.0
1957	6043.58	5859.28	96.9
1958	14540.80	13952.43	96.0
1959	25390.17	24520.45	96.6
1960	27432.59	26645.65	97.1
1961	29775.65	29775.65	100.0

1961 given in Table VII is based on catches of fish between ages 6 and 10 only; however, as shown in the 4th column, age-groups older than 10 contribute very little to the estimate in the later years.

For the 1949–55 year-classes we may also plot estimated "virtual population" abundance against the estimated abundance of their parental stock,

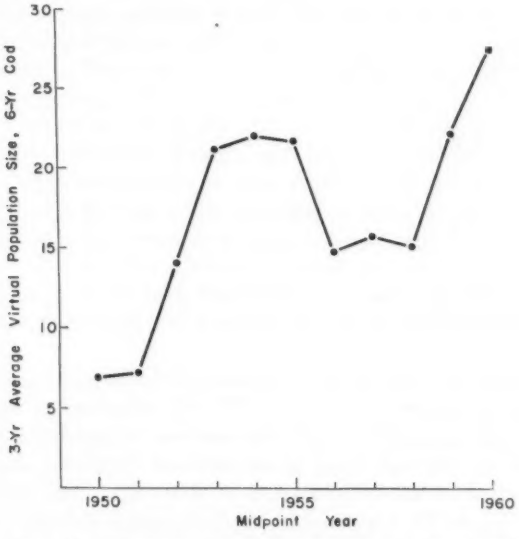

FIG. 10. Three-year running averages of the virtual population size estimates at age 6.

the latter being simply the catch-per-effort figure in weight of fish 6 years old and older. Age-at-maturity data show that the resulting figures approximate weight of mature fish landed. This results in a stock recruitment relationship shown in Fig. 11. Although it is based on data for a few years only, the plot in Fig. 11 supports our conclusion based on Fig. 10 suggesting that recruitment has gone up or that at the past levels of fish stocks in the Gulf there is an inverse relationship between the size of the stock and its filial population.

Growth and Production

In assessing the production from a stock of fish the rate of growth is of primary importance. In Table VIII we have given the estimated average weights of cod at age for the years 1949–52 and 1955–65. From these figures average growth rates may be obtained. These have been calculated for ages 0–6 and between ages 6 and 10 for each year-class. Data for older ages (10+) were not used because of high variability due to small sample sizes. The average growth up to age 6 has been calculated by taking the natural logarithm of the weight at age 6 and dividing the logarithm by 6. The average growth between ages 6 and 10 is calculated from

$$\frac{ln\,W_9 + ln\,W_{10} - ln\,W_6 - ln\,W_7}{6}$$

where $ln\,W_9$ is the natural logarithm of the average weight at age 9. This method of averaging gives the average exponential rate of growth per year. It amounts to the same as plotting the log weights against age and then calculating the slope of the line of best fit to the points; the fitting of the line to arrive at the slope is termed non-parametric (Madansky, 1959).

The resulting average growth rates have been plotted against estimated population densities in Fig. 12 and 13. All points given are 3-year running averages. As the index of the population density, the catch per effort in weight at age 6 and at ages 6–16 has been used in comparison with rates of growth up to age 6 and rates of growth between ages 6 and 10 respectively.

Both Fig. 12 and 13 suggest an inverse relationship between rate of growth and density of stock. The correlation coefficients are $r = -.623$ and $r = -.558$. At 9 degrees of freedom the 5% significance point is at $r = \pm .602$; that is, one of the correlation coefficients is significant, the other just below the significance level.

It is of interest that the growth rates up to age 6 and between ages 6 and 10 are inversely correlated with $r = -.629$ and 9 degrees of freedom. This is in keeping with the suggested inverse dependence of growth on the population density; that is, at the high level of recruitment observed in the few recent years, the growth up to age 6 has been slow. After age 6 fish have been subject to intensive fishing which has reduced the population density and resulted in faster rates of growth. This suggests at least a partial independence of trophic or other population interaction between large and small fish.

TABLE VIII. Mean weight at age of ICNAF Div. 4T cod, 2nd and 3rd quarters combined; 1953–54 based on inadequate sampling.

	Age (*years*)														
Year	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16+
1949			1.62	2.07	2.85	3.64	4.69	5.56	6.50	9.19	10.08	11.63	14.43	17.10	8.39	29.57
1950			1.82	2.39	2.47	3.36	3.74	4.25	5.33	5.95	7.11	12.23	12.71	7.55	10.27	8.83
1951			1.41	2.26	2.78	3.80	4.51	5.16	5.82	7.27	7.90	11.02	10.04	11.76	13.30	13.12
1952			1.65	1.95	2.78	3.82	4.86	5.14	5.44	6.79	6.59	8.91	9.77	11.80	11.14	13.21
1953			1.26	2.05	2.72	3.63	5.25	6.03	6.62	7.58	6.53	8.19	10.78	13.21	12.23	12.19
1954			1.48	1.70	2.33	3.31	4.17	5.31	5.26	6.14	6.33	8.22	8.36	5.90	5.72	–
1955			1.81	2.68	2.88	4.09	5.69	7.70	9.55	10.22	8.97	11.89	14.91	13.25	18.49	12.84
1956			–	2.12	3.68	4.29	5.31	7.87	9.92	10.82	12.23	11.89	14.43	14.19	14.53	9.92
1957			1.98	2.27	3.03	4.76	5.31	7.09	9.48	11.89	13.07	12.06	15.37	11.26	14.05	26.60
1958			–	2.02	2.86	3.92	5.00	5.95	7.24	9.12	9.74	13.85	13.34	13.39	21.18	18.78
1959			1.38	1.83	2.47	3.29	4.25	5.51	6.73	7.90	9.33	12.49	24.13	23.12	19.06	–
1960			1.69	1.73	2.33	3.35	4.34	5.61	7.70	7.36	10.70	11.26	11.06	10.58	17.65	23.12
1961			–	1.64	1.99	2.74	3.82	4.86	8.26	7.52	8.91	8.12	12.75	14.05	15.62	17.10
1962			0.94	1.40	1.88	2.40	3.33	4.45	4.95	7.30	12.98	8.46	15.57	28.93	27.34	32.06
1963			1.98	1.43	1.73	2.09	2.74	3.90	5.56	8.16	7.68	14.14	8.06	16.93	24.13	16.45
1964			1.30	1.37	1.85	2.19	2.67	3.36	4.67	7.87	9.92	13.07	19.36	14.57	14.82	23.31
1965			1.04	1.42	1.68	2.09	2.64	3.15	4.15	5.28	8.26	10.34	4.09	29.32	17.87	18.89

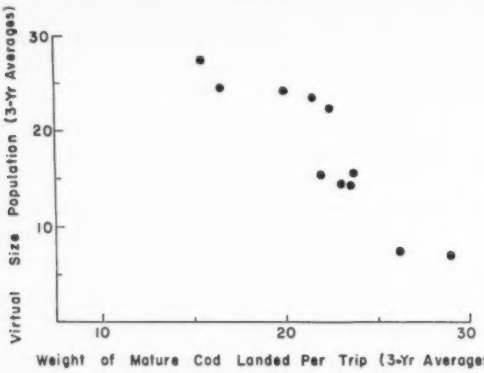

FIG. 11. Stock-recruitment relationship; virtual population estimates at age 6 plotted against weights of 6- to 16-year-old cod landed per trip 6 years earlier. Figures given are 3-year running averages.

To assess the effects of changes in rate of growth and recruitment on the yield from the stock and on its production, we have calculated the biomass elaborated by the stock. If there had been no changes in these parameters, the biomass elaborated by the stock, or what is termed production from the stock, should be reduced proportionately with the reduction in the population size by the fishery. Our calculations show, however, that the production has in fact increased.

The production has been estimated by multiplying the catch per effort in numbers at age for ages 6 and up each year by the estimated rate of growth (in weight at age) from that year to the next. The 3-year running averages

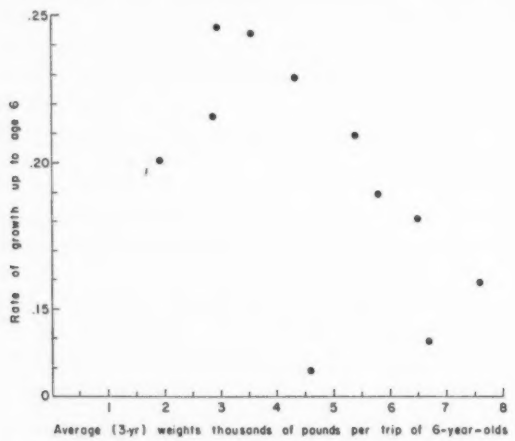

FIG. 12. Average growth rates up to age 6 plotted against estimates abundance in weight at age 6. All figures are 3-year running averages.

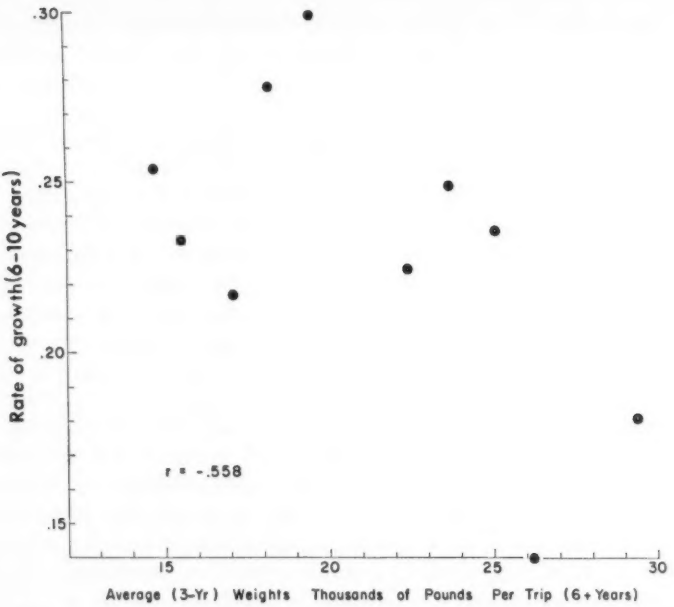

FIG. 13. Average growth rates of 6- to 10-year-old fish plotted against estimated abundance of 6- to 16-year-old fish. Figures given are 3-year running averages.

of the resulting estimates are shown in Table IX. On account of the unreliable growth data for 1953–54 only one 3-year running average with midpoint at 1950 (using growth data for 1949–50, 1950–51, 1951–52) can be calculated for the earlier period. The production figure for 1950 is lowest, suggesting that

TABLE IX. Three-year running averages of the relative biomass elaborated (i.e. production) per year.

Midpoint	Production: relative increase in weight
1950	2450
–	–
1957	3382
1958	2793
1959	2830
1960	3177
1961	3285
1962	2631
1963	3998
1964	4110

the combined effect of changes in growth and recruitment has resulted in an increase in the production from the area.

DISCUSSION

The foregoing studies may be reviewed in the light of the ICNAF Mesh Assessment Report published in 1962 (Beverton and Hodder, 1962). This report evaluated changes expected in the yield from fish stocks in the ICNAF area and in the Gulf of St. Lawrence cod stock in particular with reference to change in mesh size from the existing $4\frac{1}{2}$ inches to 4 or to 5, $5\frac{1}{2}$, and 6 inches. Minor benefits in total landings, varying between 1 and 3%, were predicted by an increase in mesh size and a small loss of about 6% was predicted if the mesh size were to be reduced to 4 inches. These predicted changes were based on calculations which assumed that a change in mesh size will not have any effect on the recruitment, growth, or natural mortality rate.

In the Gulf of St. Lawrence cod fishery the mesh size was changed in 1957. Just prior to that there had been an increase in the level of fishing effort due to a gradual build-up in the Canadian fleet and in fishing of the stock by European countries beginning about 1952. An increase in the mesh size has a tendency to reduce the effect of fishing on the stock by delaying the average age of first capture. In a sense, it is comparable to a reduction in the fishing effort on fish near the average age of first capture. This effect of the increase in the mesh size was more than counterbalanced by increases in the fishing effort. In fact, even the average age at first capture in the landings has declined. Hence the predominant feature of the fishery since 1947 has been an increased fishing at earlier ages notwithstanding the mesh size increase.

In reviewing the earlier assessment, it is important to compare the actual population changes with the premise on which that assessment was based, i.e. fixed rates of recruitment, growth, and natural mortality. No new data are available on natural mortality rate.

There is little doubt that the recruitment has been progressively higher in the more recent years and is generally higher than in the earlier years. Figure 11 suggests in fact that the higher population densities in the earlier years (1949–53) may have had a depressing effect on the recruitment and that the increased recruitment in recent years may have been related to the reduction of the cod stock, concurrent with and apparently resulting from the increased fishing. There has also been a gradual cooling of the average water temperature during much of this same period, which might in turn be expected to contribute to an increase in cod production (Martin and Kohler, 1965). However, there was no noticeable correlation between average water temperature (Apr.–Oct., Entry Island data from L. M. Lauzier, personal communication) and strength of year-class as measured at age 6 for 1943–59 year-classes. At any rate, the hydrographic change has been slight and does not appear to be related directly to the growth rate changes which are known to have been responsive to food abundance changes. It is reasonable to suppose that at least part of this recruitment effect may similarly have resulted from food and population density interactions and thus be related to the fishery effects.

In general, it is expected that a lower population density would give rise to increased growth and vice versa. Our observations of the Gulf cod stock appear to confirm this. A higher rate of recruitment in the recent years seems to have resulted in a lower rate of growth up to age 6 whereas the increased fishing and concomitant lower population densities at fishable sizes seems to have resulted in an accelerated rate of growth of older fishes sustaining the fishery, i.e. between ages 6 and 10. The total effect of these changes appears to have led to an increased production and yield from the stock.

We can only speculate on the reasons for the recruitment changes and their implications for management of the Div. 4T cod stock. It is not without importance to note two features: first, the large fish which formerly sustained the fishery were to a large extent fish (herring) feeders. These have now been virtually eliminated from the population, at no apparent sacrifice to yield. The second feature about food relations is that the large cod diet also consisted of substantial amounts of smaller food items, such as crustaceans and molluscs, the same crustacean foods which are the principal food of the smaller fish. Paloheimo and Dickie (1965) suggested that small fish are more efficient grazers of food and use it more efficiently for growth as well. We could speculate that the population response, resulting in larger total production and yields since 1953, has been partly a result of the sharp decrease in the average fish sizes, due to fishing, involving changes in the utilization of the basic food supply.

In conclusion, we may state unequivocally that the expected benefits of past and future mesh increases predicted by the yield-per-recruit (Beverton et al., 1962) calculations, could not have been realized in the presence of the observed growth responses and recruitment changes in the Div. 4T cod stock. As noted earlier, the predominant feature of this fishery has been increased fishing and an effective drop in age at first capture. In such a situation the yield-per-recruit model suggested a possible decline in productivity as defined earlier. In fact, productivity of the stock (Table IX) appears to have increased, attributable in large part to an increased recruitment. But concurrent with changes in recruitment and in fishing there were also changes in rates of growth, related possibly to abundance and food interactions. These other changes were such that had the recruitment been stable their effects would still have resulted in increased production per recruit for increased fishing and effective lowering of the age of first capture. These trends are opposite to those predicted by the model. Our study therefore suggests the increase in the mesh size in the Gulf fishery effected in 1957 was justified only on account of the elimination of the high discards of smaller cod. It cannot be justified on the basis of biological responses observed in the population nor on the basis of a simple "savings effect" as suggested in per-recruit models.

Were the compensatory changes observed in the Gulf cod stock a result of the fishery effects, as we suggest here, any future increase in the mesh size would tend to lead to lowering of the production from the stock and to losses for the fishery. On the other hand, whether an actual decrease in the mesh

size or an increase in the effective effort could give even large compensatory production changes favourable to the fishery cannot be well established without further research to determine growth efficiencies of different sized cod, cost of reproduction, and the role of competition and predation among the pre-commercial sizes.

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NOTES

Effects of Diurnal Fluctuations of Dissolved Oxygen on the Growth of Brook Trout[1]

The primary purpose of this study was to measure the effects of diurnal fluctuations of dissolved oxygen on the growth of *Salvelinus fontinalis* (Mitchill) brook trout. Previous work has shown that both constant low oxygen levels (e.g. Davison et al., 1959; Herrmann et al., 1962) and diurnal fluctuations of dissolved oxygen (e.g. Fisher, MS, 1963; Doudoroff and Warren, 1965) usually restrict salmonid growth and development. Doudoroff and Warren (1965) and Doudoroff and Shumway (1967) recently reviewed the state of our knowledge of the dissolved oxygen requirements of fishes. Bouck and Ball (1965) found that 8 hr exposure per day for 9 days to oxygen concentrations of approximately 3.5 mg/liter altered the protein composition of the blood of bluegills and largemouth bass but not of yellow bullheads. This response may or may not reduce the survival and growth of the species.

Methods.— Yearling brook trout were obtained from the Burlington Fish Hatchery, Burlington, Connecticut, 7–14 days prior to testing. They were held in 10 C water and fed commercial dry pellets once a day.

The experimental apparatus, showing two test chambers (one for experimental and the other for control fish) and one water reservoir, is diagrammed, in part, in Fig. 1. One yearling trout was transferred to each of 9–10 test chambers (3.8-liter glass jars fitted with rubber stoppers and immersed in an insulated tank) 24 hr before the tests began. Water temperatures were maintained between 8.4 and 11.7 C by refrigeration units.

Tap waters were passed through a deionizer and held in 600-liter insulated tanks; a standard complement of salts was added to the waters (Lennon and Walker, 1964). Total alkalinity was 32.6 (27.0–64.0) mg/liter; extreme values were caused by a miscalculation of the capacity of two of the reservoirs that resulted in the addition of more than the prescribed weight of salts. This error was rectified after the first test. Water temperatures in three insulated tanks were controlled by refrigeration units; waters in a fourth tank were pumped or siphoned two or more times daily into the tanks with temperature control and replaced. The addition of large volumes of water (about 250 liters) each morning into the reservoir system resulted in temperature changes of 1.1–2.3 C depending on ambient air temperatures. Water temperatures returned to "normal" in 2–5 hr, the time required depending on ambient air temperature. The small volume of water added during the day resulted in temperature changes of 0.1–0.2 C. Waters were pumped from one of the three controlled

[1]Contribution #275, Storrs Agricultural Experiment Station.

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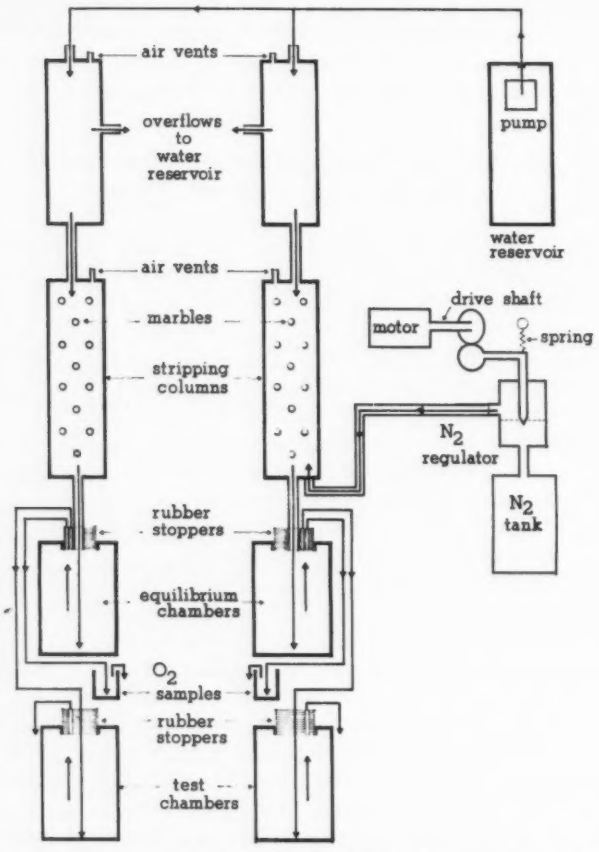

FIG. 1. Diagram of the apparatus used to measure growth of fish exposed to constant and fluctuating oxygen levels. One test vessel is shown for each equilibrating chamber. Arrows show direction of water and nitrogen flow.

temperature reservoirs to stripping columns (marble filled 10 × 122-cm glass cylinders) from which they flowed to equilibrating chambers and then to test chambers. About half of the test chambers received waters having a dissolved oxygen content of approximately air saturation (about 11 mg/liter) and the others received waters having diurnal fluctuation of dissolved oxygen. Capillary tubes, of approximately the same diameter, in the outflows of the equilibrating chambers maintained a fairly constant flow of waters to the test chambers. Water flow to test chambers receiving high oxygen levels was reduced on two tests to conserve water.

Nitrogen was introduced at the base of one of the stripping columns to control the concentration of dissolved oxygen. Oxygen was displaced while

water flowed down the column, the amount displaced being controlled by manipulation of the rate of nitrogen and water flow. Nitrogen flow occasionally had to be adjusted, usually when the nitrogen tank was almost empty. A device was constructed by the Research Shop of the University of Connecticut that controlled automatically the daily passage of nitrogen to the column by alternately opening and closing the passageway between the nitrogen tank and the column. This control resulted in high oxygen levels from 10:00 AM to 4:00 PM and low concentrations from 6:00 PM to 8:00 AM. Dissolved oxygen was measured at irregular intervals by the Alsterberg modification of the Winkler method (American Public Health Association et al., 1965). Four tests were conducted between January 1966 and February 1967. Oxygen levels fluctuated between approximately 11 mg/liter and 5.3, 3.6, 3.5, and 2.0 mg/liter respectively.

Fish were fed 1–2 g of a commercial fish food once a day after being introduced in the test chambers, usually during the time when oxygen levels were high in all chambers. Test chambers were cleaned every 2–4 days and exhaust tubings were usually cleaned every day. Fish were weighed every 7–11 days on a standard torsion balance to the nearest 0.1 g on the first test and on a direct reading balance to the nearest 0.05 g on all subsequent tests (fish and water were "poured" out of the vessel; fish were not anesthetized). Results were analyzed graphically and by analysis of covariance.

Results.— Oxygen fluctuations from approximately 11.0 to 5.3, 3.6, and 3.5 mg/liter depressed growth of yearling brook trout and most fish were unable to tolerate oxygen fluctuations to 2.0 mg/liter (Table I, Fig. 2). Analysis of covariance revealed there was a difference ($P < 0.01$) in growth between constant oxygen levels and fluctuating oxygen levels. There were no differences in growth among oxygen levels fluctuating to 5.3, 3.5, and 3.6 mg/liter. Differences in growth between individuals exposed to the same oxygen concentration increased with time, e.g. extremes of 91–107% initial body weight measured on the 10th day increased to 73–195% by the 70th day between control fishes and 87–101% on the 10th to 68–111% on the 70th day between experimental fishes.

Fish were observed feeding 5–30 days after being introduced in the test chambers, most before 10 days. Because some of the fish that were not observed feeding did gain weight, dried food must have been eaten after it sank to the bottom of the test vessel. There was no relationship between the time feeding was observed and dissolved oxygen levels. Fish observed feeding when oxygen levels were low usually did not feed with the same vigor as they did when oxygen levels were high. This reaction varied with the individual.

Discussion.— Growth of the yearling brook trout was lower for both fluctuating and high oxygen levels than was growth of other salmonids exposed to similar oxygen levels (Davison et al., 1959; Herrmann et al., 1962; Fisher, MS, 1963). Swift (1963) reported that the growth of underyearling brown trout (*Salmo trutta*) was not significantly affected by 5 weeks of exposure to oxygen levels of 5.0 mg/liter at a temperature of 16 C, but did not state

TABLE I. Summary of experimental conditions and growth of the fish.

	Test 1		Test 2		Test 3		Test 4	
Period of test:	Jan. 9, 1966 to Feb. 28, 1966		Oct. 11, 1966 to Dec. 20, 1966		Jan. 6, 1967 to Jan. 7, 1967		Jan. 7, 1967 to Feb. 3, 1967	Jan. 7, 1967 to Jan 27, 1967
No. of days:	50	50	70	70	1	1	28	21
No. of fish (*one per vessel*)	5	4	9[a]	9[a]	9	9[b]	6	4
Avg and range of oxygen (*mg/liter*)	10.6(10.3–10.8)	10.6–5.3 (5.2–5.5)	10.7(10.3–11.1)	10.7–3.6 (2.4–4.0)	11.0(10.9–11.1)	11.0–2.0 (1.9–2.1)	10.7(10.2–11.0)	10.7–3.5 (2.3–4.1)
Avg and range of water flow (*ml/min*)	43.4 (32.0–64.0)	43.4 (32.0–64.0)	23.2 (17.2–62.4)	43.6 (36.8–52.0)	35.0 (32.0–37.0)	34.0 (28.0–50.0)	23.2 (17.2–39.2)	43.6 (13.0–62.0)
Avg and range of weight on various days (g)	0 26.00 (21.3–28.9)	25.22 (14.5–38.9)	0 20.19 (16.75–26.80)	20.88 (15.00–25.80)	0 34.27 (13.15–49.65)	33.47 (20.80–48.70)	0 31.08 (13.50–49.65)	30.69 (20.80–40.40)
	11 25.08 (19.9–30.7)	24.48 (15.6–36.1)	10 19.82 (15.65–26.05)	19.37 (13.90–23.35)			7 30.26 (13.30–48.50)	29.05 (19.95–38.25)
	20 29.05 (22.0–34.3)	26.48 (17.7–39.9)	20 21.42 (15.85–26.55)	19.79 (14.10–24.60)			14 30.63 (13.50–51.00)	28.53 (19.65–37.20)
	27 30.73 (24.4–36.0)	26.06 (16.7–41.0)	31 21.76 (14.75–28.10)	18.89 (13.65–24.30)			21 31.28 (13.30–54.90)	27.84 (19.55–36.30)
	34 31.55 (25.7–37.5)	25.84 (18.2–41.3)	41 21.88 (13.40–28.30)	17.58 (11.95–22.20)			28 33.53 (13.00–58.60)	
	42 33.95 (30.0–42.6)	24.62 (17.4–42.7)	49 22.43 (12.95–30.80)	17.79 (11.15–23.75)				
	50 34.08 (30.3–42.8)	25.10 (18.6–44.3)	58 24.01 (13.20–32.40)	17.98 (10.95–23.40)				
			66 25.50 (12.70–35.40)	18.63 (10.60–25.75)				
			70 26.83 (12.30–38.10)	18.04 (10.90–22.75)				
Avg growth (*g per day*)	+0.162	−0.002	+0.095	−0.041			+0.088	−0.136

[a]One fish died of disease at both constant and fluctuating oxygen levels on the 54th day of the experiment.
[b]Only two fish survived the nighttime oxygen level.

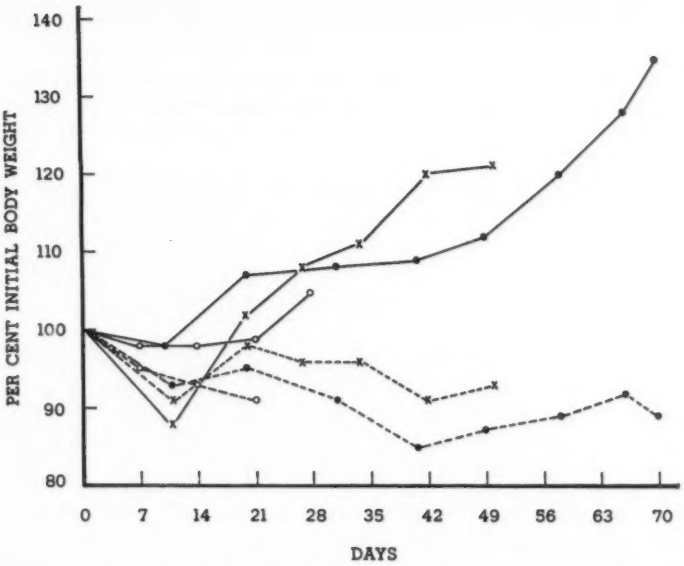

FIG. 2. Comparison of growth of yearling brook trout for all tests. Average responses at constant high oxygen levels are identified by solid lines and those at fluctuating oxygen levels by broken lines as follows: ×, 10.6–5.3; ●, 10.7–3.6; and ○, 10.7–3.5 mg/liter of dissolved oxygen.

the size of the fish. Herrmann et al. (1962) and Fisher (MS, 1963) reported that the growth of 1–6-g coho salmon (*Oncorhynchus kisutch*) was affected by low oxygen levels but some growth was obtained at oxygen levels of approximately 3.0 mg/liter at temperatures of 18 and 20 C. Growth of 2–7-g largemouth bass (*Micropterus salmoides*) as reported by Stewart et al. (1967) was not affected in waters of fluctuating dissolved oxygen at temperatures of 25–26 C as greatly as growth of yearling brook trout in waters of about 10 C.

Although many factors or interactions could have kept growth lower at all oxygen levels than reported by other investigators, I feel the interaction between size of fish and size of test chamber was the most important because most individuals (both constant and fluctuating oxygen levels) lost weight until they became acclimated to living and feeding in a 3.8-liter glass jar. The rate of water flow at low oxygen levels was probably adequate to supply the respiratory requirements of the fish. Using appropriate data interpolated and extrapolated from Beamish (1964) and the most restrictive combination of low oxygen and low rate of water flow there was enough oxygen present to supply the respiratory needs (halfway between standard and active) of the fish (gill efficiencies of 34, 31, 31, and 53% were required at the lowest oxygen levels measured in tests with average low oxygen levels of 5.3, 3.6, 3.5, and 2.0 mg/liter respectively).

Diet has not been controlled in most studies of the effects of low dissolved oxygen levels on growth. Fisher (MS, 1963) performed one test in which coho salmon were fed a "restricted" diet at constant oxygen levels from 3.0 to 18.1 mg/liter. He observed little difference in growth at all oxygen levels, possibly some effect at 3.0 mg/liter, although growth was much less than on the uncontrolled diet.

Wide variation of growth among individual brook trout exposed to the same, or approximately the same, oxygen levels has also been noted by other investigators. Herrmann et al. (1962) and Stewart et al. (1967) reported wide average differences in growth of coho salmon and largemouth bass at approximately the same oxygen levels in different experiments.

This study was supported, in part, by funds from the McIntire-Stennis Cooperative Forestry Research Program, P.L. 87-788, through the Storrs Agricultural Experiment Station, University of Connecticut. Messrs R. Voyer and D. Dorfman assisted with some of the tests; Dr J. J. Lucas advised regarding the statistical analysis of data. Fish were provided by the Connecticut State Board of Fisheries and Game.

Fisheries Management
The College of Agriculture
The University of Connecticut, Storrs, Conn.

WALTER R. WHITWORTH

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Design of a Controlled-Environment Incubator for Small Marine Fish Eggs

Studies of the effects of environmental factors on the incubation of marine fish eggs (Alderdice and Forrester, 1968) required controlled-environment incubators (Fig. 1, 2) which could be used in factorial arrays. These incubators each hold four subsamples of eggs through which water of controlled characteristics is perfused at a known rate.

The main construction materials for the incubators are molded polystyrene sheet and tubing, unplasticized polyvinyl chloride pipe fittings, polyethylene tubing connectors, and monofilament nylon mesh. The basic materials require only cutting, turning, and solvent welding in fabrication.

Newly fertilized eggs are dispensed volumetrically into the incubation baskets, and the baskets are closed and inserted into the manifold, or body, of the incubator (Fig. 1). The four separate compartments of the incubator are made watertight. O-rings located above and below a flange on the top of each incubation basket (Fig. 1, sect. R-R) form a seal with the top of the manifold when the threaded cap of each compartment is tightened down against the basket. Compartment tubing leads are then attached in series between compartments (Fig. 2). A continuous flow of water is provided through the four compartments of each incubator. The series flow arrangement was accepted to overcome the problem of providing independently controlled water flows to the four compartments of as many as 16 incubators in an experimental array. A possible bias resulting from this arrangement is averaged out for each incubator by rotating the water inlet and outlet connections to the four incubation baskets through one position daily.

Constant perfusion rates have been maintained readily with the use of Watson-Marlow Flow Inducers. These "peristaltic" type pumps were supplied with special platens which allow up to four incubators to be operated by each pump. Silicone tubing is used at the pump head because of its inertness and, particularly at low temperatures, for its flexibility and elasticity.

The incubators are submerged in individual tanks. Water in each tank (40 liters) is controlled with respect to salinity, temperature, and dissolved oxygen, and is renewed every 4 days. Water enters the suction line to a pump through a glass-wool filter located in the bottom of each tank. It is returned to the appropriate tank incubator and incubation baskets by pumping, to be discharged again into the tank. Water tempering equipment ensures the circulation of tank contents.

The incubators have been used for studies of development of marine teleost eggs of about 1 mm diam. Subsamples of about 500 eggs of this size provide a single layer of eggs in the incubation baskets. At intervals, the baskets are removed from the incubator manifolds for microscopic examination

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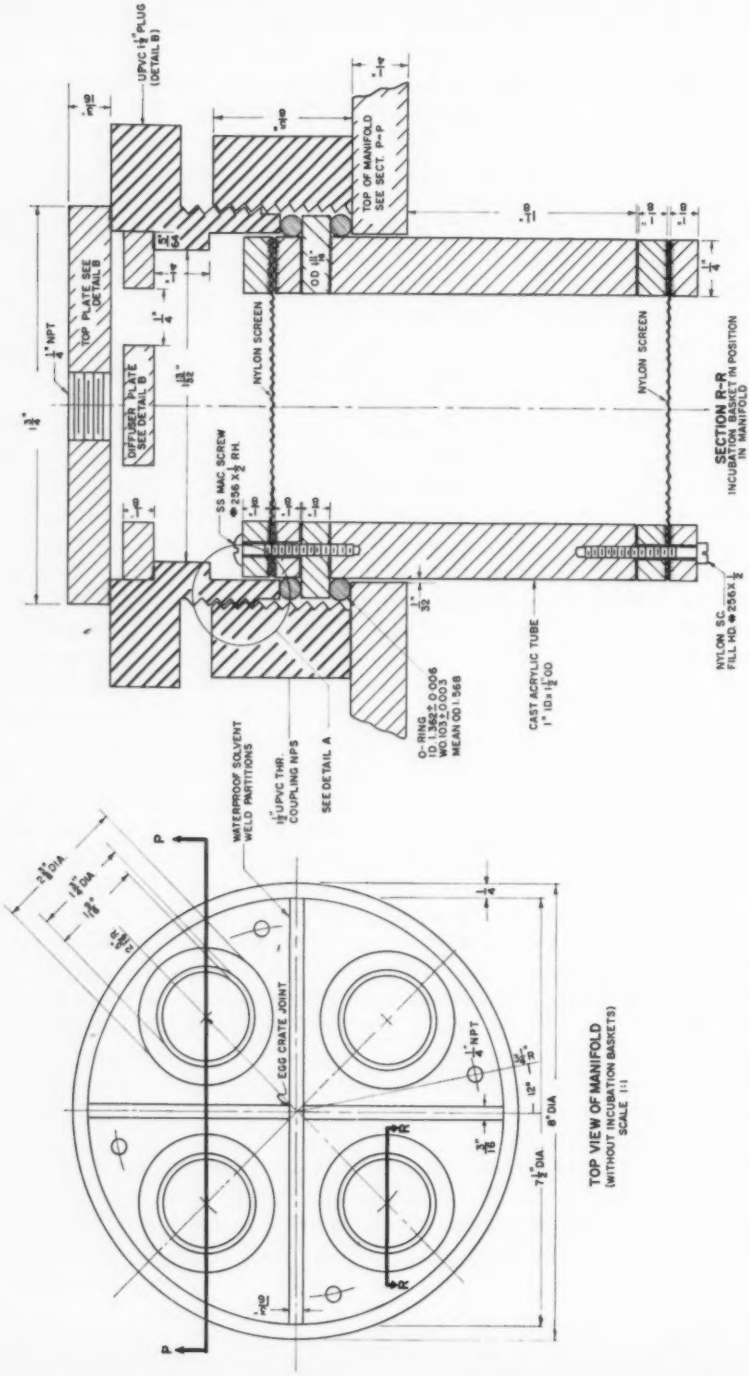
UPVC 1½" PLUG (DETAIL B)
TOP OF MANIFOLD SEE SECT P–P
TOP PLATE SEE DETAIL B
DIFFUSER PLATE SEE DETAIL B
NYLON SCREEN
NYLON SCREEN
SS MAC SCREW ⌀ 256 x ½ RH.
SECTION R–R
INCUBATION BASKET IN POSITION IN MANIFOLD
SCALE 4:1
NYLON SC. FILL HD ⌀ 256 x ½
CAST ACRYLIC TUBE 1" ID x ½" OD
O-RING ID 1.362 ± 0.006 WD 0.103 ± 0.003 MEAN OD 1.568
SEE DETAIL A
1½" UPVC THR COUPLING NPS
WATERPROOF SOLVENT WELD PARTITIONS
EGG CRATE JOINT
TOP VIEW OF MANIFOLD
(WITHOUT INCUBATION BASKETS)
SCALE 1:1

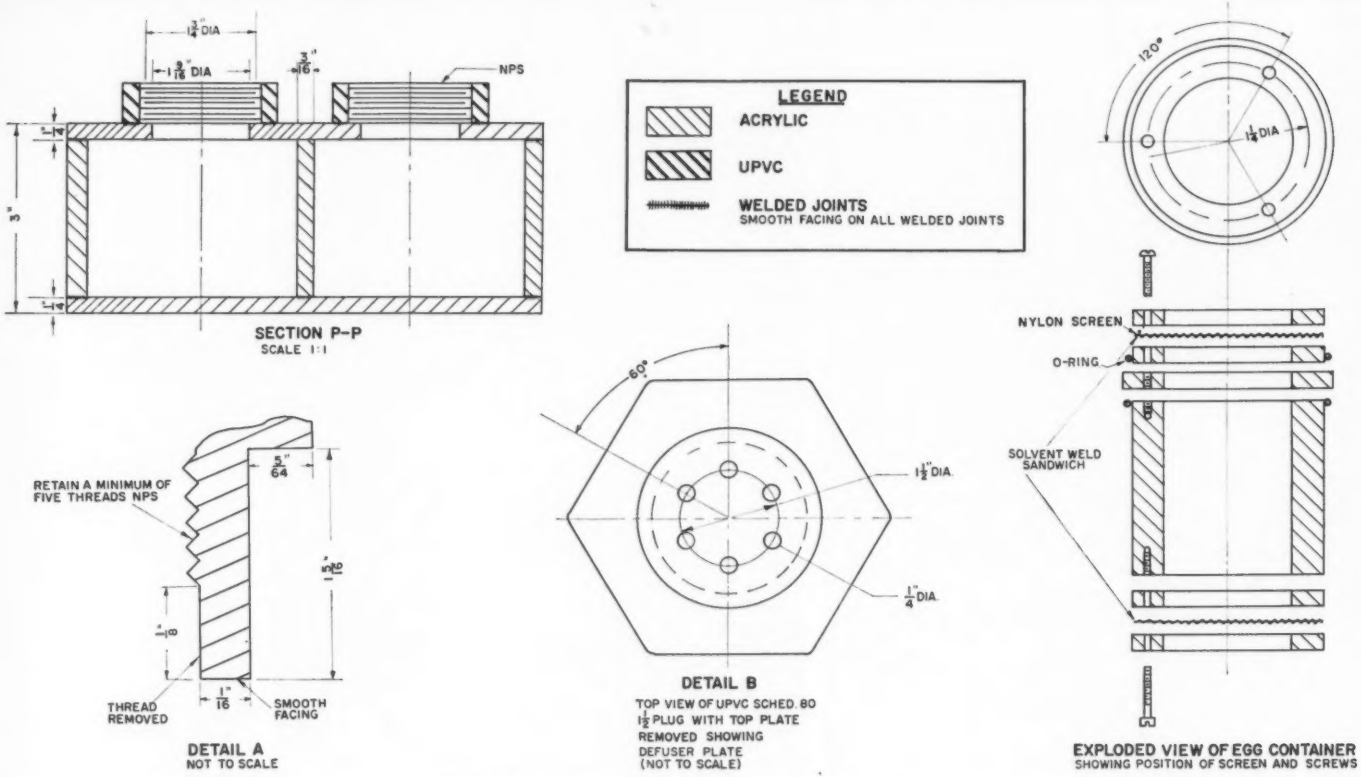

FIG. 1. Dimensions and construction details of incubator.

of egg development. At such times dead eggs or hatched larvae are removed with finely drawn transfer pipettes.

Two types of nylon mesh have been employed in the construction of the ends of the incubation baskets. These measure about 2.1 and 3.8 meshes/mm (Nitex No. 308, 175). The former has been used for larvae of 4.0 mm average length. The latter is more appropriate for smaller larvae, 2.5–3.0 mm average length.

Perfusion velocities through the incubation baskets have been maintained at approximately 2000 cm/hr by using $\frac{1}{8}$ inch inside diam $\times$ $\frac{1}{4}$ inch outside diam (3.18 mm ID $\times$ 6.35 mm OD) silicone tubing at the pumps. The incubators may be used as respirometers by connecting inlet and outlet tubes together to provide a closed-cycle circulation. In this manner oxygen consumption rates of developing eggs have been estimated. Subsequently, mass transfer calculations provide estimates of perfusion velocities at which rate of oxygen uptake of the eggs is independent of rate of oxygen transport. Calculations indicate that apparent velocities of the magnitude employed (1960–2200 cm/hr) are well in excess of those at which rate of development might be influenced by rate of oxygen delivery to the eggs.

The system described has been used primarily in detecting the effects on egg development of differences in characteristics of the perfusing medium. Where experimental conditions have been most favourable, hatches up to 94% have been achieved.

Partial list of suppliers of parts: Plastic fabrication: Capilano Engineering Ltd., Vancouver, B.C.; Watson-Marlow Flow Inducers: Foxcroft Associates, Toronto, Ont.; PVC fittings: Grinnell Co. of Canada, Vancouver, B.C.; polyethylene fittings: Cole-Parmer Instrument and Equipment Co., Chicago, Ill.; silicone tubing: Dow Silastic Medical Grade, Dow Corning Corp.; nylon mesh: Nitex, B. and S. H. Thompson and Co. Ltd., Montréal, Qué.; nylon machine screws: Nortech, Don Mills, Ont.; O-rings: Parker Seal Co., Culver City, California (part No. 2-126).

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

D. F. Alderdice
F. P. J. Velsen

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(Fig. 2 *opposite*)

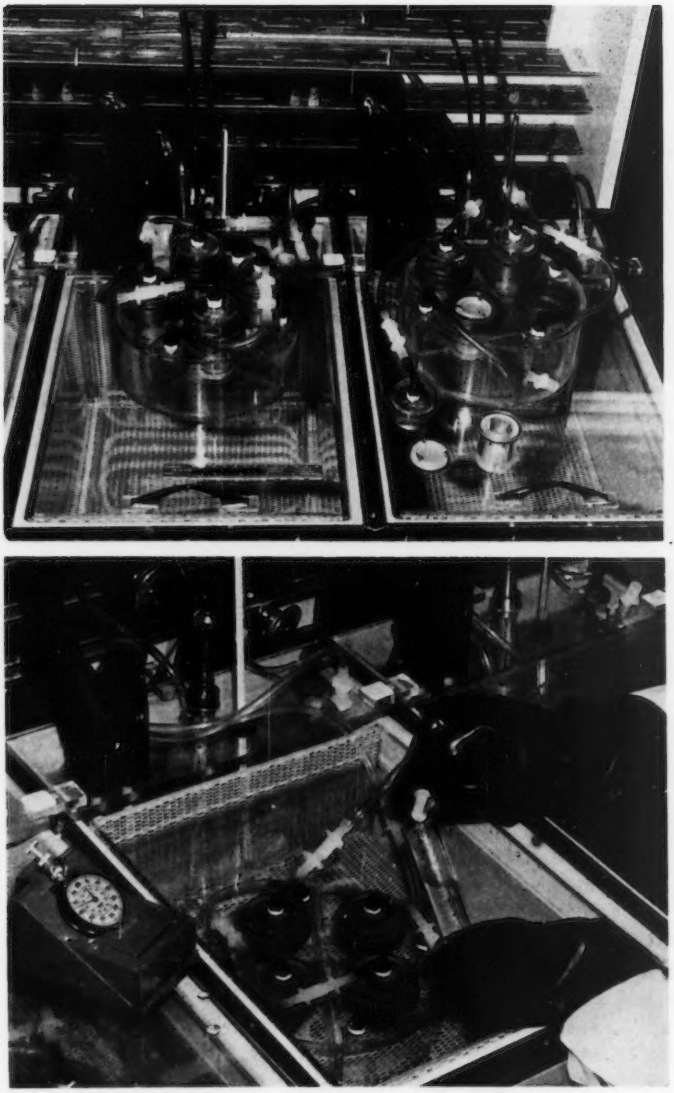

FIG. 2. *Top*, general view of incubators. The one on the left is in use; that on the right is partly dismantled to show components. *Bottom*, incubator in use. One of the two plastic tubes at the rear of the tank (far right) is the suction line to the pump (below tank). The other tube is the return line to the incubator. The compartments of the incubator are joined in series by short lengths of tubing and plastic "quick disconnects." The operator is checking the perfusion rate.

Behaviour of Herring Schools in Response to a Midwater Trawl

During midwater trawl trials, incidental observations were made on the behaviour of herring schools in response to the net. These observations were facilitated by an echo-sounder transducer attached to the headline primarily to provide information on the height of the mouth opening and on the distance of the trawl from the bottom.

The echo-sounder traces frequently showed that, as the net sank approaching a school lying on or near the bottom (Fig. 1*a–j*), the fish tended to avoid the net. The height of the school diminished noticeably (Fig. 1*b*) in many cases without compensatory increase in the apparent density of the school. The school tended to remain 1–1.5 fath below the leadline, producing a clear area on the echo-sounder trace between the leadline and the top of the school (Fig. 1*c*). In some schools this distance could not be maintained and fish were seen to enter the mouth of the net (Fig. 1*d*). Once within the net mouth the school was apparently not swept immediately back into the codend but continued swimming near the mouth. This was shown both by observations on the echo sounder and by the distributions of catches in trawls where the body of the net was fitted with fine-meshed covers.

On one occasion (Fig. 1*e–j*), echo-sounder observation demonstrated particularly clearly the presence of fish swimming in the mouth of the No. 4 trawl (Barraclough and Johnson, 1960). When the net was towed clear of the bottom, it was noticed that at speeds of about 2 knots (600 rpm) fish appeared in the mouth and spilled out below the leadline (Fig. 1*f*, *h*, *j*) but if speed was increased to about 2.5 knots (700 rpm) the fish soon disappeared from the mouth of the net. When speed was decreased again the fish echoes reappeared. This procedure was repeated three times with the same results. The last time, preparatory to hauling, the fish that escaped were observed sounding rapidly. Somewhat similar observations of fish escaping the net were made on other occasions when towing speed was reduced prior to hauling.

In a series of tows with a small conical trawl (Barraclough and Johnson, 1960) fitted with fine-meshed covers over the body, the catch increased with the quantity of fish in the area and was generally higher at towing speeds of 3.5 or more knots than at lower speeds (Table I). The proportion of the catch retained in the codend, though ranging from 19 to 85%, generally increased as the size of the catch increased (Table II). More fish escaped through the sides of the net than through the top. The proportion which escaped through the bottom was nearly always small. A smaller series of tows with a No. 4 net (Barraclough and Johnson, 1960) similarly covered yielded the same type of results. The presence of a considerable proportion of the catch in the body covers shows that herring were not immediately swept back into the codend. At least in schools lying near the bottom herring apparently did not try to

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TABLE I. The dependence of catch (in numbers of fish) of the conical net on quantity of fish in the area and speed of tow.

Qualitative estimate of fish in the area	Speed of tow						
Engine (*rpm*):	600	650	700	750	800	850	900
Knots:	2.40	2.65	2.95	3.25	3.50	3.75	4.00
Above avg	353	–	2896	484	4170	–	1542
Avg	–	–	499	–	1964	1391	–
Below avg	–	44	18	38	164	–	535

TABLE II. Percentages of catches of various sizes escaping to the sides, top, or bottom of the body, or retained in the codend, of two types of trawl.

Catch range (numbers)	No. of hauls	Per cent of catch in							
		Covers on							
		Side		Top		Bottom		Codend	
		Avg	Range	Avg	Range	Avg	Range	Avg	Range
		Conical trawl							
0–500	12	43	6–76	13	0–30	3	0–9	41	19–85
500–1,000	4	41	27–56	15	8–25	2	1–3	42	27–64
1,000–2,000	2	24	20–28	11	5–16	2	2–3	60	48–73
2,000–3,000	3	13	10–15	14	12–17	5	2–8	68	66–70
3,000–4,000	1	12	12	4	4	12	12	72	72
		No. 4 trawl							
0–500	2	67	54–79	11	0–22	3	0–6	19	18–20
500–1,000	3	33	19–40	5	0–9	14	1–20	48	43–53
1,000–2,000	0	–	–	–	–	–	–	–	–
2,000–3,000	2	16	14–19	10	0–20	38	9–68	35	13–58
9,000–10,000	1	4	4	2	2	0	0	94	94

escape by sounding but rather by turning to one side or the other, or to a lesser extent by swimming upward. When the net was well clear of the bottom, however, echo-sounder observations showed that escaping herring did sound (Fig. 1).

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

F. H. C. TAYLOR

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BARRACLOUGH, W. E., AND W. W. JOHNSON. 1960. Further midwater trawl developments in British Columbia. Bull. Fish. Res. Bd. Canada, No. 123. 45 p.

(FIG. 1 *opposite*)

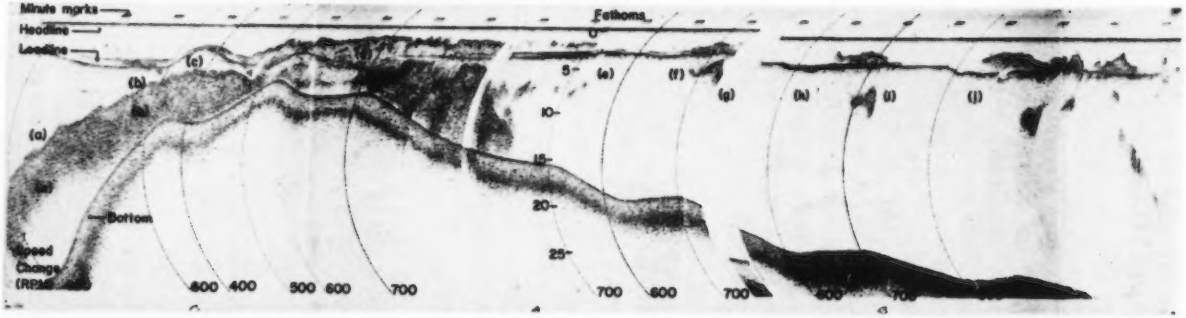

FIG. 1. Record from an echo-sounder transducer on the headline of No. 4 midwater trawl showing: (*a*) herring school on bottom; (*b*) height of school decreasing as net approaches; (*c*) clear zone between leadline and school; (*d*) herring entering mouth of net; (*e, g, i*) mouth of net clear of fish at speeds of 2.5 knots (700 rpm); (*f, h, j*) fish in and below mouth of net at reduced speed of 2 knots (600 rpm). NOTE: The transducer was fastened to the headline of the net, which was thus the zero line from which depths were measured. As the net sank the distance to the bottom became less, and with reference to the zero line (the headline of the net) the bottom appeared to rise.

Taylor — J. Fish. Res. Bd. Canada

A Postulated Effect of Growth on Retention Time of Metabolites[1]

We have previously suggested that the rate of radionuclide excretion may be quite closely correlated with body weight (Eberhardt and Nakatani, 1967; Eberhardt, 1967). Such a relationship has been widely reported on an interspecies basis, and seems an obvious corollary of the well-known dependency of many metabolic functions on body size (Brody, 1945).

Our purpose here is to suggest some simple models for radionuclide uptake and excretion which also take into account the effects of growth, anP to reexamine some previously reported data in light of these models. We believe a really adequate treatment of the subject will require both more elaboration of the model, and additional experimentation. The present results should, however, provide a basis for designing further experiments and, hopefully, stimulate the reappraisal of existing data.

Exposition of the model.— The underlying model for uptake and retention is here assumed to be:

$$\frac{dy}{dt} = \lambda(t) - \mu(t)y$$

where $y = y(t)$ = body burden at time t; $\lambda(t)$ = intake at time t; $\mu(t)$ = rate of excretion at time t. For present purposes we neglect any "compartment" effects, and suppose that $\lambda(t)$ and $\mu(t)$ pertain to a single "pool" of radionuclide. A further simplification is introduced by supposing growth to be exponential, i.e.

$$W = W_0 e^{kt}$$

where $W = W(t)$ = weight at time t; W_0 = initial weight; k = a constant.

The exponential relationship is, of course, satisfactory only over a restricted portion of the growth curve. Assumption of exponential growth seems realistic for the data used for illustration here, and simplifies exposition of a model. The use of more complex growth models seems reasonably straightforward, but the explicit form will depend on the organism under consideration, and the resulting differential equations may require solution by numerical methods.

The postulated relation of excretion rate to body weight is:

$$\mu = AW^{-B}$$

where $\mu = \mu(t)$ = excretion rate at time t; $W = W(t)$ = body weight at time t;

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A,B = constants. In studies of metabolic functions, B is often in the neighborhood of 0.8 (Brody, 1945). Eberhardt (1967) obtained an estimate of about 0.6 for excretion of ^{137}Cs by humans. We suppose B will definitely lie in the range $0 \leq B < 1$.

Assuming exponential growth, excretion rate may be expressed as a function of time as:

$$\mu = AW^{-B} = A(W_0e^{kt})^{-B} = AW_0^{-B}e^{-Bkt}.$$

Radionuclide uptake is here considered to be approximated by a continuous process. The degree of approximation should be satisfactory if "biological half-times" are reasonably long relative to the interval between feedings. The usual experimental protocol prescribes administration of radionuclides in proportion to body weight:

$$\lambda(t) = b\ W(t).$$

Hence transforming to units of time yields:

$$\lambda(t) = bW = bW_0e^{kt}.$$

We can now recast the basic differential equation as:

$$\frac{dy}{dt} + [AW_0^{-B}e^{-Bkt}]\ y = bW_0e^{kt}.$$

Attempts at explicit solution require evaluation of:

$$\int \exp\left\{ -\frac{AW_0^{-B}}{Bk}\ e^{-Bkt} \right\} e^{kt}\ dt$$

which does not have a solution in closed form, but can be calculated numerically. Such calculations call for better estimates of the constants than we now have, so we turn to some other aspects of the model.

Turning to the excretion phase (i.e., assuming no further intake of radionuclide), a solution is readily obtained:

$$\frac{dy}{dt} = -\mu(t)y = -[AW_0^{-B}e^{-Bkt}]y$$

and, since

$$\frac{d\log(y)}{dt} = \frac{1}{y} \cdot \frac{dy}{dt}$$

we have:

$$\log y = -AW_0^{-B} \int e^{-Bkt} dt + C$$

or:

$$y = y_0 \exp \left\{ - \frac{AW_0^{-B}}{Bk} [1 - e^{-Bkt}] \right\}$$

where y_0 is the body burden at the beginning of the excretion process. When the exponent, Bkt, is "small," the equation becomes approximately:

$$y = y_0 e^{-AW_0^{-B}t}.$$

Thus, retention follows the usual exponential decay model if growth proceeds slowly, or observations are limited to short time spans. For actively growing animals and longer time spans, semi-logarithmic plots of body-burden against time should yield a curve, rather than the straight line of a "single-component" loss. Further, as t becomes large, the model postulated here prescribes approach to an asymptotic body burden. However, we caution that actual realization of such a consequence depends on persistence of exponential growth.

Transforming back to a weight scale we find:

$$y = y_0 \exp \left\{ - \frac{AW_0^{-B}}{Bk} [1 - (\frac{W_0}{W})^B] \right\}.$$

Thus it is possible to examine plots on a scale of either time or weight.

An example.— Some data previously reported by Nakatani (1966) may serve to illustrate an application of the above results. Figure 1 shows the uptake and retention of ^{65}Zn by rainbow trout (*Salmo gairdneri*) fed 0.1 μCi per gram of fish per day, plotted on a time scale. An insert (Fig. 1) shows the exponential nature of the growth exhibited by these fish. The fish were 52 weeks of age at the beginning of the experiment.

In view of the variability in body burden between individuals at each measurement (fish were sacrificed for radioanalysis in this experiment), and the likelihood of an initial "fast component," we hesitate to suggest that Fig. 1 provides any support for an hypothesis of non-linear excretion (on the semi-logarithmic plot). In fact, the original analysis of the data suggested an effective half-life (long-term retention) of 134 days, on the basis of linear regression analysis of logarithms of individual body burdens against time.

The model here postulated may, however, be rather more satisfactorily illustrated by a semi-logarithmic plot of body burden against weight (Fig. 2). There seems to be both a distinct relationship between body burden and

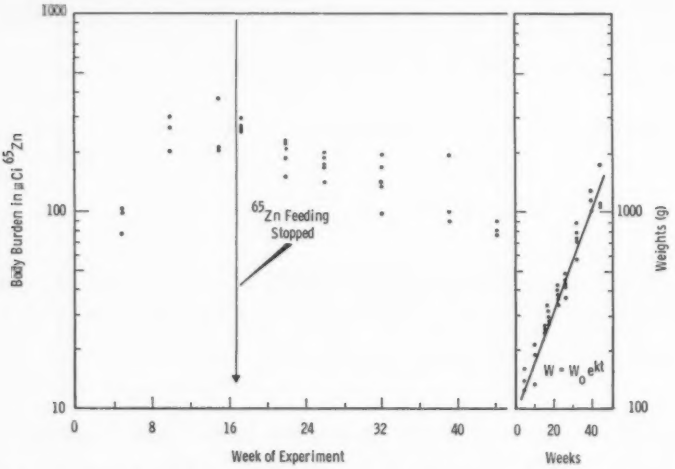

FIG. 1. Uptake and retention of ^{65}Zn by rainbow trout fed 0.1 μCi per gram of fish per day, plotted on a time scale. Insert shows the exponential nature of the growth exhibited by these fish.

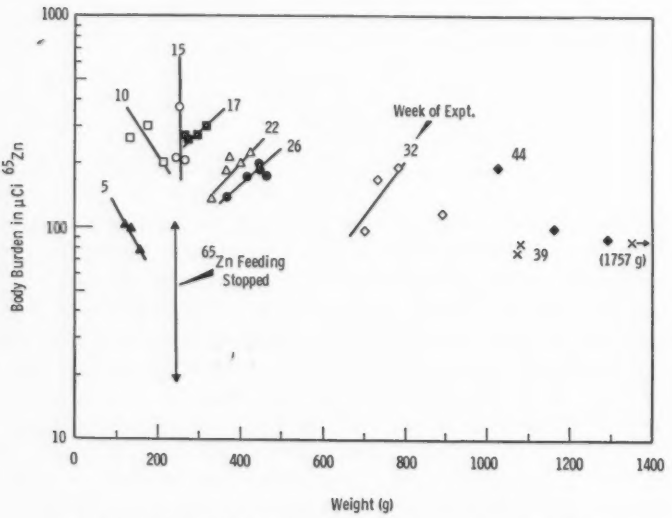

FIG. 2. Semi-logarithmic plot of body burden against weight.

weight in many of the sets of fish analyzed (numbered by week of killing) and a strong suggestion of an asymptotic behavior of the mean body burden. Straight lines in the figures are "eye-fitted," for illustrative purposes.

Appraisal of the change of the "within-group" relationship between body burden and weight up to the time feeding of ^{65}Zn was discontinued is complicated by the lack of a simple expression for the uptake portion of the curve. In general, a fish that began the experiment at a relatively low (or high) weight might be expected to hold roughly the same relative position throughout. Hence one could argue that a single body burden vs. weight curve might simply be displaced to the right or left, depending on the initial weight of the individual fish.

Discussion.— We do not presume to argue that the evidence of Fig. 1 and 2 is conclusive as to the nature of the models best representing the underlying relationships. The data do serve to illustrate our postulated model, and it would seem that a more detailed analysis might provide some additional evidence. However, our purpose in the present report is mainly that of postulating an important effect of growth on apparent half-time. Also, further analysis of the ^{65}Zn data seems to us a somewhat dubious proposition since the experiment was not designed for the purpose at hand. In particular, we propose that the obvious procedure is to "whole-body count" individual fish, thus securing a complete curve for each individual. We further note that radionuclide intakes might be proportioned to weight individually, whereas these fish were fed (by capsule) amounts proportional to mean weight of the entire surviving group.

From the point of view of construction of better models, we note that many useful results concerning metabolism and growth of fish are available in a comprehensive set of papers by Paloheimo and Dickie (1965, 1966a, 1966b). A full analysis needs also to take into account the "compartmental" effects, although we suspect these may be more readily handled than one might at first suppose, as a consequence of the usual allometric and similarity relations (i.e., the relative compartment sizes may remain nearly constant over much of the growth period). Stochastic aspects constitute another wide field of inquiry, considering that certainly W_0, B, and k can be expected to vary appreciably in practice, and that various other factors will influence their behavior (e.g., temperature, nutritional level).

It may be worth noting that the present model provides another prospect for failing to observe a simple exponential loss in "half-time" experiments. Three others were suggested elsewhere (Eberhardt and Nakatani, 1967) as: (1) excretion as a sum of exponentials, as frequently postulated; (2) excretion rates changing in time — the "power-law" relation often used in analyzing behavior of bone-seeking radionuclides; (3) behavior of body burdens averaged over a population of individuals having different excretion rates.

Biology Department
Battelle Memorial Institute
Pacific Northwest Laboratory, Richland, Wash.

L. L. EBERHARDT
R. E. NAKATANI

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Ichthyophonus Infection in Yellowtail Flounder (*Limanda ferruginea*) off Nova Scotia

A systemic disease in yellowtail flounder from western Sable Island Bank (43°50′N lat, 61°40′W long) was first observed in July 1966. The disease was characterized by the presence of white cysts on the liver and other internal organs. These cysts were readily visible to the naked eye. The proportion of infected fish was about 20% (Table I). Microscopic examinations showed that the disease is caused by a fungus similar to or indentical with *Ichthyophonus* (= *Ichthyosporidium*). This is the first recorded observation of the disease in the yellowtail flounder. Since the time of its first observation three sampling surveys (September 1966, February and May 1967) have confirmed that the disease is still present.

TABLE I. Percentages of yellowtail flounder from western Sable Island Bank infected in various degrees by *Ichthyophonus*. Results are based on gross examination of 146 fish caught in February 1967 by otter trawl.

Degree of infection	% infested
None: no cysts visible in liver — other organs normal	0.0
Light: liver spotted with cysts — individual cysts discrete	15.1
Moderate: more than half of liver surface encysted	4.8
Heavy: liver surface completely matted with cysts	0.7
Total	20.6

Ichthyophonus is known to be capable of invading a wide range of hosts. Although the primary host of the disease in the western North Atlantic is believed to be the herring *Clupea harengus* (Sindermann, 1958), natural infections have also been reported for populations of winter flounder (Ellis, 1928) and alewives (Fish, 1934). A number of freshwater fish and a few marine invertebrates are apparently also susceptible to infection by this parasite.

Diseased fish exhibited no external evidences of infection. In field examinations, various degrees of infection of the liver (Fig. 1) were noted. Parasitic cysts of all sizes up to about 2 mm were also found on most of the internal organs including the heart, intestinal tract, spleen, and kidneys. The cysts appeared to be identical in all organs. The heart and liver were the sites of heaviest infection. In some cases, the heart was completely covered by the cysts. Such heavy infection of the heart strongly suggests a high mortality among diseased fish, and surveys (Royce et al., 1959) show that yellowtail landings from the Sable Island region have decreased in recent years. Cysts were occasionally observed on both the inside and outside walls of the stomach

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and intestine. In two heavily diseased fish the fungus produced gross distortion and enlargement of the kidney. Infestation of the spleen and gall bladder was only rarely observed. In many fish, mesenteries also had lesions, most frequently on or near blood vessels. No diseased tissue was observed in the body musculature.

Fisheries Research Board of Canada
Biological Station, St. Andrews, N.B.
P. M. POWLES[1]

Department of Zoology
McGill University, Montreal, P.Q.
D. G. GARNETT

Osborn Laboratories of Marine Sciences
New York Aquarium, Brooklyn, N.Y.
G. D. RUGGIERI, S.J.
R. F. NIGRELLI

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(FIG. 1 *opposite*)

[1]Present address: Department of Biology, Queen's University, Kingston, Ont.

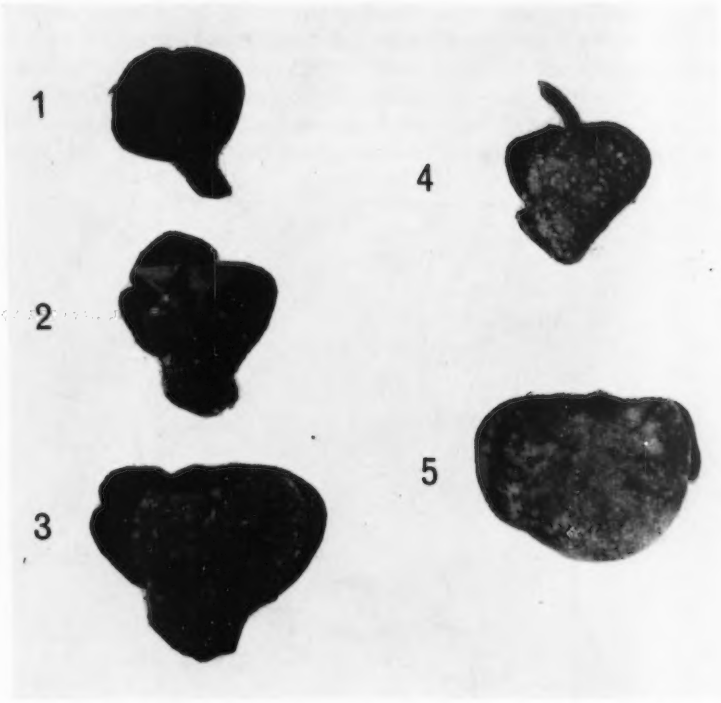

FIG. 1. Formalin-preserved livers of yellowtail flounder showing different degrees of *Ichthyophonus* infection. Cysts appear as white spots (1) which eventually cover the whole liver (5).

A First Report of Chum Salmon Fry Feeding in Fresh Water of British Columbia

Chum salmon fry, *Oncorhynchus keta* (Walbaum), have been recorded feeding in fresh water in the Paratunka (Synkova, 1951) and Amur (Levanidov and Levanidova, 1957) river systems of the USSR. Large chum fry have been reported from the Ishikari River system of Hokkaido (Sano, 1966) but no details were given regarding food eaten by fry in fresh water. According to Neave (1966), feeding of chum fry in fresh water has not been reported in British Columbia and to my knowledge only a few workers (Tyler and Bevan, 1964; Walker and Lister, MS, 1965) have reported the capture of North American chum fry which have fed intensively in fresh water.

Examination of juvenile salmon from the Cowichan River, British Columbia (Fig. 1) showed that some chum fry, in association with chinook (*O.*

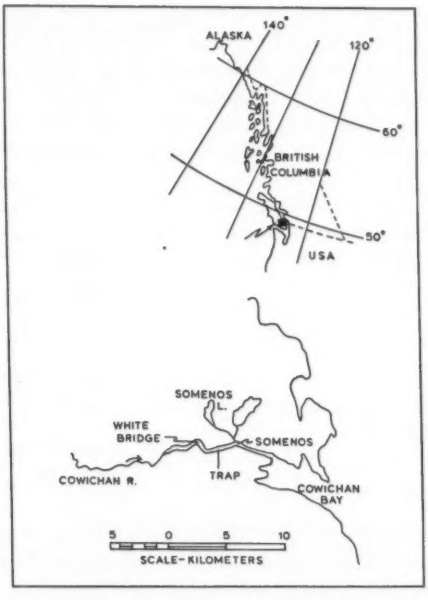

FIG. 1. Location of the study area and sampling stations on the Cowichan River.

tshawytscha) and coho (*O. kisutch*) salmon, had not only been feeding in fresh water but were larger than expected from measurements of chum fry which move directly to sea.

From Cowichan Lake (elevation 162 m) the Cowichan River flows eastward 52 km to enter the ocean. The stream gradient below the White Bridge

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is not as steep (average gradient 1.6 m/km) as the upper part of the river (average 3–5 m/km). The level of the Cowichan River at Somenos (Fig. 1), although affected by tides during periods of high river discharge (usually in late fall or winter months), is above the limits of saltwater intrusion.

Traps were used in the Cowichan River to collect fry moving downstream at night; at Somenos and the White Bridge, fry were captured with a fine-meshed seine.

Length and weight measurements were usually taken from samples of anaesthetized fry. About 5% should be added to the length of preserved fry for direct comparison with live lengths (Parker, 1963). Fork lengths were recorded to the nearest millimeter; individual weights were taken to the closest 0.05 g after blotting the specimens to remove excess water.

Circuli were counted from scales of 47 fry collected near Somenos and preserved in formalin in 1966. Scales were obtained from a specific area between the dorsal and adipose fins, just dorsal to the lateral line.

Volumes of stomach contents from Cowichan River chum fry, preserved in formalin, were measured by displacement in the barrel of a 1.0-ml hypodermic syringe (graduated to 0.01 ml). Organisms were examined under a binocular dissecting microscope and recorded by major classification groups.

Analysis of stomach contents of chum fry revealed the general occurrence of chironomids in the diet of fish collected at Somenos and the White Bridge (Table I). In addition to chironomids, fry from Somenos contained cladocerans.

TABLE I. Stomach analysis of chum salmon fry collected in 1966 in the Cowichan River.

Station:	Somenos	White Bridge
Date:	April 28	May 4
Sample size:	25	25
Fork length (*mm*)		
Mean	52	37
Range	48–58	35–40
Volume stomach contents (*ml*)		
Mean	0.06	<0.01
Range	0.03–0.09	<0.005–0.02
% frequency of occurrence		
Chironomidae larvae	92	96
Simuliidae larvae	4	8
Daphnidae	100	–
Bosminidae	80	–
Ephemeroptera nymph	8	12
Ephemeroptera winged	–	4
Plecoptera nymph	12	12
Trichoptera nymph	–	4
Diptera winged	4	–
Hymenoptera	–	4
Copepoda juveniles	8	–
Nematoda	–	20
Mean no. Chironomidae per fish	6.7	13.5

Some had eaten Bosminidae and all contained representatives of *Daphnia* sp. Somenos Creek is a slow-moving stream with abundant aquatic vegetation and therefore it seems likely that zooplankton from this area contributed to the diet of fry below the Somenos–Cowichan confluence.

Fry collected at Somenos in 1966 were much larger than fry taken in traps in 1965 (Table II). Fry as large as 67 mm and 2.5 g were caught at Somenos and fish were collected as late as June 9. Chum fry from the White Bridge were more similar in size to fry collected with traps.

TABLE II. Size of Cowichan River chum salmon fry compared with data from other investigations.

Sample		No. of fish	Fork length (*mm*)		Weight (g)	
			Mean	Range	Mean	Range
Cowichan River						
Traps	1965 Mar. 27–Apr. 17	83	36.7[a]	32–41		
	May 16–17	25	37.5	36–43	0.35	0.30–0.45
Somenos	1966 April 28	46	55.3	38–63	1.40	0.30–2.15
	May 4	20	48.9	39–66	0.95	0.40–2.35
	May 12	10	53.6	45–67	1.26	0.65–2.50
White Bridge	1966 May 4	25	38.7	36–41	0.38	0.30–0.50
Hooknose Creek	1961 April 30	50	37.2	33–40[b]	0.285	0.20–0.34[b]
Big Qualicum River						
	1960 May 1–15	190[c]	36.6[d]	31–40		
	1961 May 1–15	205	39.0	35–50		
	June 1–15	122	40.7	36–61		
	1962 May 1–15	>100	38.8	35–43	0.39	

[a]Fry preserved in 10% formalin.
[b]R. R. Parker, personal communication.
[c]Sample size in all years from personal communication or MS reports of Canada Department of Fisheries.
[d]Probably from preserved fry.

Range in number of circuli was derived by examining six scales from each fish. Fry of 35–38 mm were without scales; 42–64 mm fry had 1–5 scale circuli. Chum fry of 39–41 mm were not represented in samples for scale analysis.

Very few workers in North America have presented length and weight information for chum fry (Table II). Size of downstream migrant fry caught in traps in the Cowichan River appears similar to data reported for the Big Qualicum River (Walker and Lister, MS, 1965) and Hooknose Creek (Parker, 1963). A progressive increase in average length from mid March to mid June has been reported for fry moving to sea from the Qualicum River by Walker and Lister (MS, 1965). But late (June 1–15) migrant fry from the Qualicum were

much smaller in average length than chum fry collected near Somenos, in the lower Cowichan River (Table II).

In a length-frequency diagram, Tyler and Bevan (1964) show large chum fry (range 58–83 mm) from "a late season catch in the Nooksack River," Washington. It was indicated that chum fry delayed migration and grew in the Nooksack River before undergoing downstream migration.

The importance of Chironomidae in the diet of chums was mentioned by Synkova (1951) and chironomids occurred in 71% of the chum fry examined from Amur River tributaries (Levanidov and Levanidova, 1957). The principal food of chum salmon fry from the west coast of Kamchatka (Sano, 1966) was larvae of chironomids, larvae and adult forms of other insects, copepods, and phyllopodans.

Data from the Cowichan River showed that some chum salmon had grown considerably in fresh water before entering the sea. Some fry caught in late April and early May were comparable in size to Asian chum salmon taken in June and July in fresh water (Semko, 1954; Sano, 1966).

Fisheries Research Board of Canada
Biological Station, Nanaimo, B.C.

R. A. H. SPARROW

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Addendum: Catecholamine and Carbohydrate Concentrations in Rainbow Trout (*Salmo gairdneri*) in Relation to Physical Disturbance

Since the paper by Nakano and Tomlinson (1967) was published, two earlier publications dealing with catecholamine concentrations in fish blood, and with factors influencing those concentrations, have come to the attention of the authors. Fontaine et al. (1963) found adrenaline (AD) to be the predominant catecholamine in the blood of *Salmo salar*, and Mazeaud (1964) found that noradrenaline (NAD) predominated in the blood of carp. In our work with *S. gairdneri*, although the increase in blood AD concentration was much greater than that of NAD in response to disturbance, similar to the relation observed by Fontaine et al. in *S. salar*, nevertheless the concentrations of NAD were similar to those reported earlier for carp by Mazeaud (1964). Thus it appears that the difference between these fish resulted from a difference in quantity of AD secreted into the blood.

Fisheries Research Board of Canada
Vancouver Laboratory, Vancouver, B.C.

T. NAKANO[1]
N. TOMLINSON

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[1]National Research Council of Canada Post Doctorate Fellow 1964–65. Present address: Holy Ghost Junior College, Akita City, Japan.

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Ultrasound Backscatter in Fresh and Thawed Animal Tissue

Values of velocity and absorption of high-frequency ultrasound in animal tissue can be correlated to some extent with the quality and composition of the tissue. Although this approach has received some attention, no effective inspection method based on measurements of these parameters has been developed to date. This is partly because measurements of these values require exacting conditions and precise instrumentation, such as an accurate time base, attenuator, and measurement cell.

The authors have found that the backscatter of high-frequency ultrasound from beef and fish tissue differs markedly for fresh tissue and for tissue which has been frozen and then thawed. Initial tests have shown that the backscatter is highly dependent on the rate of freezing, and the number of times a sample has been frozen.

The measurement of backscatter is straight forward. For the measurements, a pulsed transducer ringing at 3.16 MHz for 1.5 μsec was used. A focused ultrasonic beam was directed though a water path at the immersed sample. A part of the incident beam penetrates the sample and is scattered at tissue interfaces having different acoustic impedances (equal to the square root of the product of compressibility and density). Some of the scattered energy is reflected back to the transmitting transducer which also serves as the receiver. The received echoes are then amplified, demodulated, and displayed as a function of time, time being approximately proportional to tissue depth.

The oscilloscope traces obtained, called A-scans, are shown in Fig. 1 for samples of fresh and thawed fillet of whitefish (*Coregonus clupeaformis*). The fresh sample, Fig. 1A, was scanned and then frozen at −10 C. A second fresh sample was immediately frozen at −10 C and served as a reference. After thawing at room temperature, the first sample was scanned again; this A-scan is shown in Fig. 1B.

The large pulse at the left of each scan in Fig. 1A and B is the echo from the scales of the fish. The last echo at the right is from the midline of the fish. A great increase in number and amplitude of the echoes between the scale and midline echo is evident in Fig. 1B. The mean amplitudes of these echoes, which will be referred to as tissue noise, are approximately 16 db higher than the corresponding echoes in Fig. 1A. The number of observable distinct echoes per centimetre of tissue at a particular gain and frequency is also significant. For example, a moderate freezing rate (−20 C ambient) results in numerous peaks similar to those in Fig. 1B but having considerably lower average amplitudes; a slower freezing rate (−5 C ambient) results in slightly fewer distinct peaks but larger average amplitudes. The original freezing rate can thus be roughly estimated from the number and amplitude of the noise peaks. These tests were extended to the contact method (the transducer is placed in contact with the sample), and similar results were obtained.

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The exponential decay of the echo amplitudes shown in Fig. 1B and in the subsequent figures is the result of attenuation of the incident and the scattered pulses. The attenuation was measured for the fresh and thawed samples; the values were 2.3 db/cm and 4.8 db/cm respectively. A fairly good correlation of these values with the increase in the number of noise peaks is observed.

Using the same experimental procedure, tests were made on samples of beef filet mignon with the beam propagating perpendicular and parallel to the fibers of the tissue. The results are shown in Fig. 2 and 3. The large equispaced echoes at the right were caused by reverberation in a flat aluminum blate supporting the samples, which was not visible in Fig. 1 because the sample was thicker. As in Fig. 1, the thawed tissue shows a great increase in backscatter. However, the attenuation has not changed substantially for parallel incidence as can be seen by inspecting the amplitudes of the reverberation echoes in 2A and B. The measured values of absorption were 5.1 db/cm and 5.6 db/cm for the fresh and thawed tissue respectively.

The noise level in fresh beef tissue for perpendicular incidence (Fig. 3A) is seen to be much higher than for parallel incidence (Fig. 2A). This might be caused partly by acoustic impedance discontinuities between the muscle fibers.

The increase in amplitude of the echoes as a result of freezing and thawing is less pronounced for perpendicular than for parallel incidence, but the number of noise peaks is greater. Numerous small echoes can be distinguished superimposed on the large noise peaks in Fig. 3B. In addition, unlike the situation for parallel incidence, the absorption for perpendicular incidence increases from 1.8 db/cm to 5.1 db/cm. The amplitudes of the reverberation echoes reflect this increase.

The changes in backscatter are primarily due to the presence of numerous small cavities in the tissues caused by formation of ice crystals and dissolved air which comes out of solution during the freezing process. The size and number of these cavities and thus the amplitude distribution of tissue noise is highly dependent on the rate of freezing. Therefore, the noise amplitude distribution can be directly related to the "freezing history" and the general physical condition of the tissue sample. A more detailed study of the relationship of noise distribution to tissue condition is now being made.

The backscatter property described above may be applied to quality control and inspection of fish and meat. Probably the method could also be extended to food products other than animal tissue. The contact method would likely be preferred because of its simplicity in cases where good contact can be maintained.

Fisheries Research Board of Canada — M. FREESE
Freshwater Institute, Winnipeg, Man.

Photogrammetric Research — D. MAKOW
Division of Applied Physics
National Research Council of Canada, Ottawa, Ont.

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(FIG. 1–3 *opposite*)

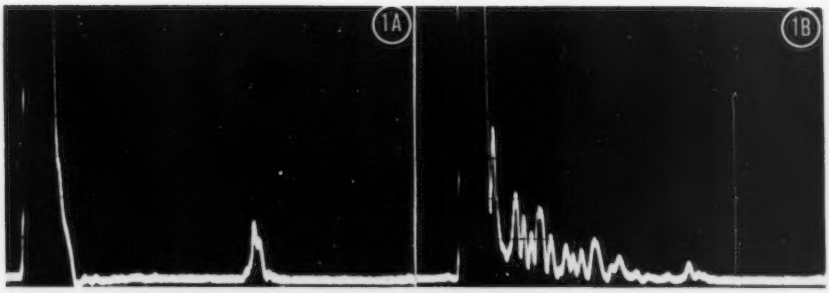

FIG. 1. A, A-scan of fresh whitefish sample. Vertical scale is in arbitrary units, horizontal is about 6 μsec/cm. B, A-scan of same sample after freezing at −10 C and thawing.

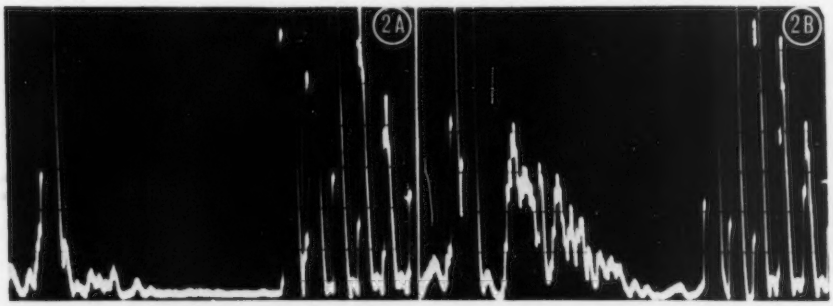

FIG. 2. A, fresh beef filet mignon, beam propagating parallel to tissue fibers. Horizontal scale is about 5 μsec/cm. B, same sample after freezing at −10 C and thawing.

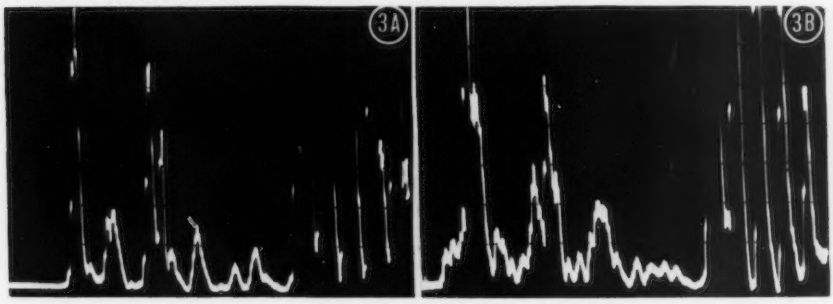

FIG. 3. A, fresh beef filet mignon, beam propagating perpendicular to tissue fibers. Horizontal scale is about 5 μsec/cm. B, same sample after freezing at −10 C and thawing.

Characteristics of Hemolymphs of *Cancer irroratus*, *C. borealis*, and *Hyas coarctatus*

The lobster pathogen *Gaffkya homari* (Snieszko and Taylor, 1947; Hitchner and Snieszko, 1947) grows as well in vitro in the hemolymph serum from *Cancer irroratus*, *C. borealis*, or *Hyas coarctatus* (Cornick and Stewart, 1968a) as it does in that of the lobster *Homarus americanus* (Rabin, 1965; Cornick and Stewart, 1968b). When *C. irroratus* was challenged by *G. homari*, the microorganism was found to be mildly pathogenic for this crab species (Cornick and Stewart, 1968a). These facts led to our comparing some of the characteristics of the hemolymphs of these local crab species with those of lobster hemolymph.

Crabs, which showed no signs of molting, were captured in and around the entrance to Halifax Harbour and were kept in tanks of free-flowing sea water at ambient temperatures similar to those of their natural habitat (3–8 C). Measurements were made within 2 weeks of the animals' capture. Hemolymph samples were drawn from the cavity covered by the membranes at the base of the crabs' legs. Methods for preparing hemolymph for free boundary electrophoretic measurements and determining protein and *p*H values were identical to those used in studies of the lobster (Stewart et al., 1966).

Trials using distilled water, NaCl solutions of 0.5, 0.85, 1, or 2% concentrations, or a mixed salt solution with or without 0.5 ml/liter Tween 80 (polyoxyethylene sorbitan monooleate) (Stewart et al., 1967a) showed that increasing salt concentrations improved the stability of crab hemocyte suspensions. The mixed salt solution plus 0.5 ml/liter Tween 80 at *p*H 7.6 proved to be the most effective diluting fluid for the electronic counting of crab hemocytes as it was for counting lobster hemocytes (Stewart et al., 1967a). All crab hemocyte counts were made by this method.

Classification of the hemocytes (fixed with 0.25% iodine) follows the nomenclature of Toney (1958). The only difference, apart from the ratios of hemocyte types, observed between the lobster hemocytes and those from the three crab species was that the crab hemocytes were slightly smaller.

The results summarized in Table I show that levels of serum protein vary considerably from species to species. They are all considerably lower than those normally found in the lobster (Stewart et al., 1967b). The hemocyte numbers, with the exception of those for *H. coarctatus*, are only slightly less than the numbers found in the lobster (Stewart et al., 1967a, b). The correlation coefficients between the serum protein levels and the hemocyte numbers, with the exception of that for the female *C. borealis* specimens, indicate a reasonably direct relationship between them. More work is required before this correlation could be considered to be of practical importance. All

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TABLE I. Hemolymph values for *Cancer irroratus*, *C. borealis*, and *Hyas coarctatus*.

Species	Sex	No. of animals	Mean body weight (g) + SD[a]	Mean hemolymph serum protein values(*mg/ml*) + SD	Mean hemocyte values/mm³ ×10⁴ + SD	Correlation coefficient (cells:serum protein)	Ratio of hemocytes[b] *Lga*:*Sga*:*Ly*	Hemolymph *p*H[c] + SD
Cancer irroratus	♂	28	179.5±34.2	23.0±10.5	2.24±1.14	0.723	16:10:1	7.38±0.1 14 ♂ at 20 C
C. borealis	♂ + ♀	32	181.8±44.2	23.8±9.7	2.02±.85	0.511	11:9:1	
	♂	18	206.2±41.6	23.6±10.1	1.79±.74	0.699		7.53±0.15
	♀	14	150.6±22.7	24.2±9.1	2.32±.88	0.366		19 ♂ at 20 C 7.49±0.11 13 ♀ at 20 C
Hyas coarctatus	♂ + ♀	20	55.8±26.8	14.6±10.1	.97±.76	0.865	14:11:1	
	♂	13	60.4±31.1	12.1±10.4	.96±.92	0.948		7.55±0.11
	♀	7	46.0±6.6	18.3±8.0	.99±.33			10 ♂ at 20 C
Homarus americanus	(For *H. americanus* hemocyte and hemolymph values see Stewart et al., 1967b.)							
	♂ + ♀	20					25:13:1	7.59±0.06[d]

[a]SD = standard deviation.
[b]*Lga* = large granule amoebocytes; *Sga* = small granule amoebocytes; *Ly* = lymphocyte (lymphoid cell type).
[c]Sea water *p*H 7.42 at 20 C.
[d]From Stewart et al., 1966 (51 lobsters).

of the foregoing hemolymph values vary widely between individuals and would be expected to change considerably with diet changes and the particular molting stage. The *p*H values for the different species are not significantly different from one another nor from that of the lobster (Stewart et al., 1966), particularly when temperature corrections are made.

Typical electrophoretic patterns of the hemolymphs are shown in Fig. 1. For each species the patterns of the serum and the oxalated plasma were similar; consequently, only the oxalated plasma patterns are shown. It was not possible to find any distinct difference that could be attributed to the disappearance of fibrinogen from the plasma. The hemolymphs tended merely to become more viscous rather than form a firm clot as does the lobster hemolymph which also suggested that if a true fibrinogen was present it was in low concentrations only. This is in marked contrast to the lobster in which fibrinogen forms 7–9% of the total plasma proteins (Stewart et al., 1966). The patterns of the three species were readily distinguishable from each other and it was not possible, from a study of mobilities, to relate the minor peaks in the pattern of one species to those of another species. In three samples of hemolymph from *C. borealis* the area of the main peak, assumed by analogy with lobster data (Stewart et al., 1966) to be hemocyanin, comprised 84–91% of the total components, in fair agreement with that found for lobster. A reliable estimate of hemocyanin concentrations was not possible for the other two hemolymphs because the colour and opalescence of the samples partially obscured the peaks assumed to be hemocyanin. The mobilities of the hemocyanins were fairly constant within a given species, but differed rather markedly from species to species (Table II). Manwell and Baker (1963) showed with starch–gel electrophoresis that this was also true for several other crab species.

No outstanding differences between the lobster and crab hemolymphs other than those connected with fibrinogen were observed. The mild path-

TABLE II. Electrophoretic mobilities[a] of the principal component in crab plasmas and sera.

	Plasma		Serum	
	Ascending	Descending	Ascending	Descending
Cancer borealis	8.04	7.55	8.04	7.74
	7.95	7.91	7.93	7.60
C. irroratus	8.46	7.72	8.60	7.83
	8.59	8.14	8.78	7.97
Hyas coarctatus	7.30	6.53	6.93	6.27
	6.93	6.43	6.90	6.35

[a]Units of mobility: $cm^2\ volt^{-1}\ sec^{-1} \times 10^{-5}$.

ogenicity of *G. homari* for one of the crab species (*C. irroratus*) has been recorded (Cornick and Stewart, 1968a) and no reason is apparent from that study or this to exclude *G. homari* as a potential pathogen for *C. borealis* or *H. coarctatus*.

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Fisheries Research Board of Canada
Halifax Laboratory, Halifax, N.S.

JAMES E. STEWART
J. R. DINGLE

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(FIG. 1 *opposite*)

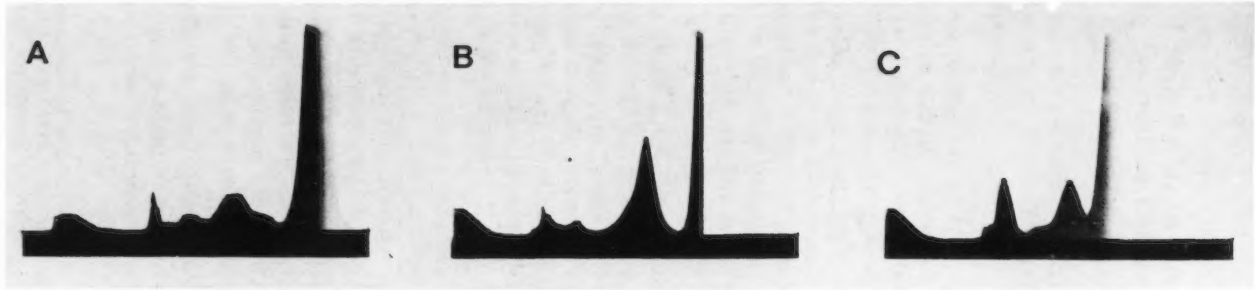

FIG. 1. Typical electrophoretic patterns of hemolymph plasmas from the three crab species (minimum of two separate determinations, each done on pooled material from 10 males). Descending patterns only; migration to the right. Buffer was potassium phosphate, total ionic strength 0.21, *p*H 7.8–8.0; temperature 1.4 C: gradient 5.1–5.2 volts/cm. A, *Cancer borealis*, 18 mg protein/ml of sample; B, *C. irroratus*, 25 mg protein/ml of sample; C, *Hyas coarctatus*, 20 mg protein/ml of sample.

Stewart and Dingle — J. Fish. Res. Bd. Canada

Induction of Skin Pallor in Juvenile Salmonids by Guanidine Compounds[1]

A great many compounds have been studied in relation to pigment changes and distribution in the skin of fishes. The guanidines have received little attention in this connection.

When eaten by young chinook salmon (*Oncorhynchus tshawytscha*) weighing 30–40 g, the drug galegine sulfate (4-methyl-3-butenyl guanidine sulfate) induced lightening of the dorsal skin color. The effect was noticeable in less than 2 hr and marked in 3 hr after one meal of a hatchery diet (Fowler et al., 1964) to which the drug had been added to a concentration of 0.06% (Fig. 1). A similar and more marked pallor occurred in less than 7 min after intraperitoneal injection of 0.02 ml of saline containing 100 μg of the drug into salmon quieted in water containing 20 mg of M.S. 222[2] (tricaine methanesulfonate) per liter (Fig. 1). The dorsal area of the head paled first in each instance.

Pallor persisted for about 2 days after a single meal containing the drug and the fish refused food during that time. They also showed a slight difficulty in maintaining a normal swimming position for several hours after the meal. Injected fish had greater difficulty maintaining equilibrium and at higher levels of the drug (200 or 300 μg) remained on their sides for some time after the injection. Injected salmon also appeared lethargic for several hours; the normal color returned in about 1 day.

Rainbow trout (*Salmo gairdneri*) weighing 10–12 g reacted similarly to injection of the drug although a larger amount per unit body mass was required and the skin color lightening was not as rapid or as marked. Trout also showed the adverse effects on equilibrium.

Frogs (*Rana pipiens*) did not show visible changes in skin color after administration of the drug either by stomach tube or by intraperitoneal injection. Polywogs (unknown species) showed no color change to a large dose given intraperitoneally (100 μg of drug in polywogs estimated to weigh 3 g).

Guanidine hydrochloride injected in isomolar amounts (to the galegine sulfate) into salmon caused a slight skin pallor. The injection of phenethylbiguanide hydrochloride, decamethylenebiguanidine carbonate, N-butyl-

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[2]Sandoz Pharmaceuticals, Hanover, New Jersey.

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biguanide hydrochloride or 1,1-dimethylbiguanide resulted in variable and equivocal findings.

University of Oregon Medical School — W. R. TODD
Department of Biochemistry, Portland, Ore. — L. E. LAASTUEN

Bureau of Sport Fisheries and Wildlife — L. G. FOWLER
Salmon-Cultural Laboratory, Longview, Wash.

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(FIG. 1 *opposite*)

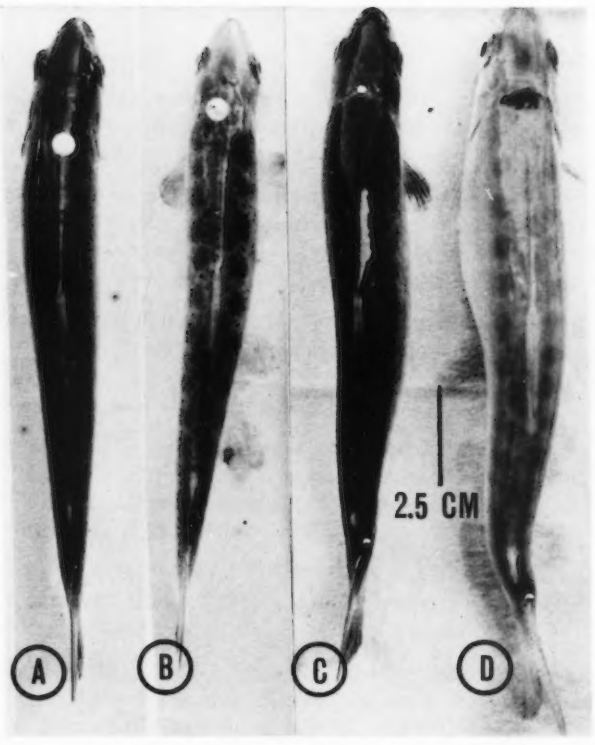

FIG. 1. Salmon A received the diet without drug. Salmon B (40 g) consumed one meal of a basal diet containing 0.06% of 4-methyl-3-butenyl guanidine sulfate (galegine sulfate) 3 hr before the photograph was taken. Drug intake estimated at 510 μg. Salmon C was injected with saline only. Salmon D (40 g) was given 0.02 ml of saline containing 100 μg of galegine sulfate intraperitoneally 7 min prior to the photograph.

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